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Published
With international search report.

(54) Title: INSECTICIDAL PROTEIN TOXINS FROM *PHOTORHABDUS*

(57) Abstract

Proteins from the genus *Photorhabdus* are toxic to insects upon exposure. *Photorhabdus luminescens* (formerly *Xenorhabdus luminescens*) have been found in mammalian clinical samples and as a bacterial symbiont of entomopathogenic nematodes of genus *Heterorhabditis*. These protein toxins can be applied to, or genetically engineered into, insect larvae food and plants for insect control.

INSECTICIDAL PROTEIN TOXINS FROM *PHOTORHABDUS*

Cross-reference to Related Application

5   This patent application is a continuation-in-part of U.S.
Patent Application Serial Number 08/743,699 filed on
November 6, 1996, which is a continuation-in-part of U.S. Patent
Application Serial Number 08/705,484 filed on August 28, 1996,
which is a continuation-in-part of U.S. Patent Application Serial
10   Number 08/608,423 filed February 28, 1996, which is a continuation-
in-part of U.S. Patent Application Serial Number 08/395,947 filed
February 28, 1995, which was a continuation-in-part of U.S. Patent
Application Serial Number 08/063,615 filed May 18, 1993. This
application is also a continuation-in-part of provisional U.S.
15   Patent Application Serial Number 60/007,255 filed November 6, 1995.

Field of the Invention

         The present invention relates to toxins isolated from bacteria
20   and the use of said toxins as insecticides.

Background of the Invention

         Many insects are widely regarded as pests to homeowners, to
25   picnickers, to gardeners, and to farmers and others whose
investments in agricultural products are often destroyed or
diminished as a result of insect damage to field crops.
Particularly in areas where the growing season is short,
significant insect damage can mean the loss of all profits to
30   growers and a dramatic decrease in crop yield. Scarce supply of
particular agricultural products invariably results in higher costs
to food processors and, then, to the ultimate consumers of food
plants and products derived from those plants.
         Preventing insect damage to crops and flowers and eliminating
35   the nuisance of insect pests have typically relied on strong
organic pesticides and insecticides with broad toxicities. These
synthetic products have come under attack by the general population
as being too harsh on the environment and on those exposed to such
agents. Similarly in non-agricultural settings, homeowners would
40   be satisfied to have insects avoid their homes or outdoor meals
without needing to kill the insects.
         The extensive use of chemical insecticides has raised
environmental and health concerns for farmers, companies that

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produce the insecticides, government agencies, public interest
groups, and the public in general. The development of less
intrusive pest management strategies has been spurred along both by
societal concern for the environment and by the development of

5   biological tools which exploit mechanisms of insect management.
Biological control agents present a promising alternative to
chemical insecticides.

Organisms at every evolutionary development level have devised
means to enhance their own success and survival. The use of

10   biological molecules as tools of defense and aggression is known
throughout the animal and plant kingdoms. In addition, the
relatively new tools of the genetic engineer allow modifications to
biological insecticides to accomplish particular solutions to
particular problems.

15   One such agent, *Bacillus thuringiensis* (*Bt*), is an effective
insecticidal agent, and is widely commercially used as such. In
fact, the insecticidal agent of the *Bt* bacterium is a protein which
has such limited toxicity, it can be used on human food crops on
the day of harvest. To non-targeted organisms, the *Bt* toxin is a

20   digestible non-toxic protein.

Another known class of biological insect control agents are
certain genera of nematodes known to be vectors of transmission for
insect-killing bacterial symbionts. Nematodes containing
insecticidal bacteria invade insect larvae. The bacteria then kill

25   the larvae. The nematodes reproduce in the larval cadaver. The
nematode progeny then eat the cadaver from within. The bacteria-
containing nematode progeny thus produced can then invade
additional larvae.

In the past, insecticidal nematodes in the *Steinernema* and

30   *Heterorhabditis* genera were used as insect control agents.
Apparently, each genus of nematode hosts a particular species of
bacterium. In nematodes of the *Heterorhabditis* genus, the
symbiotic bacterium is *Photorhabdus luminescens*.

Although these nematodes are effective insect control agents,

35   it is presently difficult, expensive, and inefficient to produce,
maintain, and distribute nematodes for insect control.

It has been known in the art that one may isolate an
insecticidal toxin from *Photorhabdus luminescens* that has activity
only when injected into Lepidopteran and Coleopteran insect larvae.

40   This has made it impossible to effectively exploit the insecticidal
properties of the nematode or its bacterial symbiont. What would
be useful would be a more practical, less labor-intensive wide-area
delivery method of an insecticidal toxin which would retain its

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biological properties after delivery. It would be quite desirous
to discover toxins with oral activity produced by the genus
Photorhabdus. The isolation and use of these toxins are desirous
due to efficacious reasons. Until applicants' discoveries, these
5   toxins had not been isolated or characterized.

Summary of the Invention

The native toxins are protein complexes that are produced and
10   secreted by growing bacteria cells of the genus *Photorhabdus*, of
interest are the proteins produced by the species *Photorhabdus
luminescens*. The protein complexes, with a molecular size of
approximately 1,000 kDa, can be separated by SDS-PAGE gel analysis
into numerous component proteins. The toxins contain no hemolysin,
15   lipase, type C phospholipase, or nuclease activities. The toxins
exhibit significant toxicity upon exposure administration to a
number of insects.

The present invention provides an easily administered
insecticidal protein as well as the expression of toxin in a
20   heterologous system.

The present invention also provides a method for delivering
insecticidal toxins that are functional active and effective
against many orders of insects.

Objects, advantages, and features of the present invention
25   will become apparent from the following specification.

Brief Description of the Drawings

Fig. 1 is an illustration of a match of cloned DNA isolates
30   used as a part of sequence genes for the toxin of the present
invention.

Fig. 2 is a map of three plasmids used in the sequencing
process.

Fig. 3 is a map illustrating the inter-relationship of several
35   partial DNA fragments.

Fig. 4 is an illustration of a homology analysis between the
protein sequences of TcbA$_{ii}$ and TcaB$_{ii}$ proteins.

Fig. 5 is a phenogram of *Photorhabdus* strains. Relationship
of *Photorhabdus* Strains was defined by rep-PCR.
40   The upper axis of Fig. 5 measures the percentage similarity of
strains based on scoring of rep-PCR products (i.e., 0.0 [no
similarity] to 1.0 [100% similarity]). At the right axis, the
numbers and letters indicate the various strains tested; 14=W-14,

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Hm=Hm, H9=H9, 7=WX-7, 1=WX-1, 2=WX-2, 88=HP88, NC-1=NC-1, 4=WX-4,
9=WX-9, 8=WX-8, 10=WX-10, WIR=WIR, 3=WX-3, 11=WX-11, 5=WX-5, 6=WX-
6, 12=WX-12, x14=WX-14, 15=WX-15, Hb=Hb, B2=B2, 48 through 52=ATCC
43948 through ATCC 43952. Vertical lines separating horizontal

5   lines indicate the degree of relatedness (as read from the
extrapolated intersection of the vertical line with the upper axis)
between strains or groups of strains at the base of the horizontal
lines (e.g., strain W-14 is approximately 60% similar to strains H9
and Hm).

10   Fig. 6 is an illustration of the genomic maps of the W-14
Strain.
      Fig. 6A is an illustration of the tca and tcb loci and primary
gene products.

      Fig. 7 is a phenogram of *Photorhabdus* strains as defined by
15   rep-PCR. The upper axis of Fig. 7 measures the percentage
similarity of strains based on scoring of rep-PCR products (i.e.,
0.0 [no similarity] to 1.0 [100% similarity]). At the right axis,
the numbers and letters indicate the various strains tested.
Vertical lines separating horizontal lines indicate the degree of
20   relatedness (as read from the extrapolated intersection of the
vertical line with the upper axis) between strains or groups of
strains at the base of the horizontal lines (e.g., strain Indicus
is approximately 30% similar to strains MP1 and HB Oswego). Note
that the *Photorhabdus* strains on the phenogram are as follows: 14
25   = W-14; Hm = Hm; H9 = H9; 7 = WX-7; 1 = WX-1; 2 = WX-2; 88 = HP88;
NC1 = NC-1; 4 = WX-4; 9 = WX-9; 8 = WX-8; 10 = WX-10; 30 = W30; WIR
= WIR; 3 - WX-3; 11 = WX-11; 5 = WX-5; 6 = WX-6; 12 = WX-12; 15 =
WX-15; X14 = WX-14; Hb = Hb; B2 = B2; 48 = ATCC 43948; 49 = ATCC
43949; 50 = ATCC 43950; 51 = ATCC 43951; 52 = ATCC 43952.
30

Detailed Description of the Invention

      The present inventions are directed to the discovery of a
35   unique class of insecticidal protein toxins from the genus
Photorhabdus that have oral toxicity against insects. A unique
feature of *Photorhabdus* is its bioluminescence. *Photorhabdus* may
be isolated from a variety of sources. One such source is
nematodes, more particularly nematodes of the genus
40   *Heterorhabditis*. Another such source is from human clinical
samples from wounds, see Farmer et al. 1989 J. Clin. Microbiol. 27
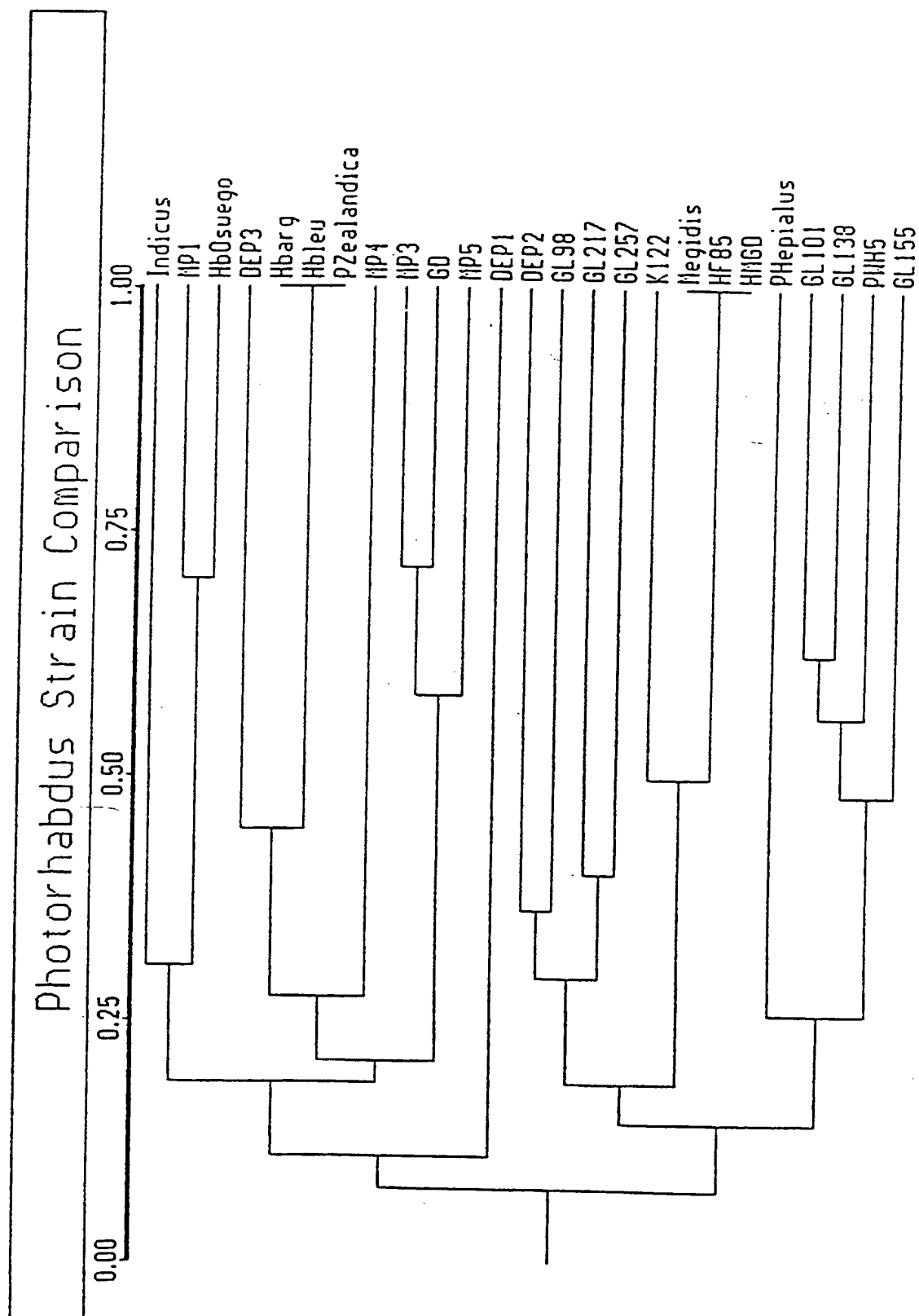
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Photorhabdus Strain Comparison

FIG. 7

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :Please See Extra Sheet.
US CL   :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

  U.S. :   435/69.1, 172.1, 172.3, 243, 252.3, 320.1, 419; 530/350, 536/23.7, 24.1; 800/205, 250

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

  APS, CABA, CAPLUS, BIOSIS, MEDLINE, GENBANK, SCISEARCH
  search terms: insecticide, protein, photorhabdus, xenorhabdus, transgenic, transformed, plant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WILSON et al. Laboratory tests of the potential of entopathogenic nematodes for the control of field slugs. Journal of invertebrate Pathology. 1994, Vol. 64, pages 182-187.	1-99
Y	CLARKE et al. Virulence mechanisms of Photorhabdus sp. strain K122 toward wax moth larvae. Journal of Invertebrate Pathology. 1995, Vol. 66, pages 149-155.	1-99
Y	VAECK et al. Transgenic plants protected from insect attack. Nature. July 1987, vol. 328, pages 33-37.	1-99

☐   Further documents are listed in the continuation of Box C.   ☐   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
		"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 AUGUST 1997	0 3 SEP 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 1/00, 1/20, 15/00, 15/09, 15/10, 15/29, 15/31, 15/82; A01G 13/00; A01H 1/00, 3/00, 4/00, 5/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 172.1, 172.3, 243, 252.3, 320.1, 419; 530/350, 536/23.7, 24.1; 800/205, 250

pp. 1594-1600. These saprohytic strains are deposited in the American Type Culture Collection (Rockville, MD) ATCC #s 43948, 43949, 43950, 43951, and 43952, and are incorporated herein by reference. It is possible that other sources could harbor

5   *Photorhabdus* bacteria that produce insecticidal toxins. Such sources in the environment could be either terrestrial or aquatic based.

The genus *Photorhabdus* is taxonomically defined as a member of the Family *Enterobacteriaceae*, although it has certain traits

10 atypical of this family. For example, strains of this genus are nitrate reduction negative, yellow and red pigment producing and bioluminescent. This latter trait is otherwise unknown within the *Enterobacteriaceae*. *Photorhabdus* has only recently been described as a genus separate from the *Xenorhabdus* (Boemare et al., 1993 Int.

15 J. Syst. Bacteriol. 43, 249-255). This differentiation is based on DNA-DNA hybridization studies, phenotypic differences (e.g., presence (*Photorhabdus*) or absence (*Xenorhabdus*) of catalase and bioluminescence) and the Family of the nematode host (*Xenorhabdus*; *Steinernematidae*, *Photorhabdus*; *Heterorhabditidae*). Comparative,

20 cellular fatty-acid analyses (Janse et al. 1990, Lett. Appl. Microbiol 10, 131-135; Suzuki et al. 1990, J. Gen. Appl. Microbiol., 36, 393-401) support the separation of *Photorhabdus* from *Xenorhabdus*.

In order to establish that the strain collection disclosed

25 herein was comprised of *Photorhabdus* strains, the strains were characterized based on recognized traits which define *Photorhabdus* and differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* species. (Farmer, 1984 Bergey's Manual of Systemic Bacteriology Vol. 1 pp.510-511; Akhurst and Boemare 1988, J. Gen. Microbiol. 134

30 pp. 1835-1845; Boemare et al. 1993 Int. J. Syst. Bacteriol. 43 pp. 249-255, which are incorporated herein by reference). The traits studied were the following: gram stain negative rods, organism size, colony pigmentation, inclusion bodies, presence of catalase, ability to reduce nitrate, bioluminescence, dye uptake,

35 gelatin hydrolysis, growth on selective media, growth temperature, survival under anaerobic conditions and motility. Fatty acid analysis was used to confirm that the strains herein all belong to the single genus *Photorhabdus*.

Currently, the bacterial genus *Photorhabdus* is comprised of a

40 single defined species, *Photorhabdus luminescens* (ATCC Type strain #29999, Poinar et al., 1977, Nematologica 23, 97-102). A variety of related strains have been described in the literature (e.g., Akhurst et al. 1988 J. Gen. Microbiol., 134, 1835-1845; Boemare

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et al. 1993 Int. J. Syst. Bacteriol. 43 pp. 249-255; Putz et al.
1990, Appl. Environ. Microbiol., 56, 181-186). Numerous
Photorhabdus strains have been characterized herein. Because there
is currently only one species (*luminescens*) defined within the

5   genus *Photorhabdus*, the *luminescens* species traits were used to
characterize the strains herein. As can be seen in Fig. 5, these
strains are quite diverse. It is not unforeseen that in the future
there may be other *Photorhabdus* species that will have some of the
attributes of the *luminescens* species as well as some different

10   characteristics that are presently not defined as a trait of
Photorhabdus luminescens. However, the scope of the invention
herein is to any *Photorhabdus* species or strains which produce
proteins that have functional activity as insect control agents,
regardless of other traits and characteristics.

15   Furthermore, as is demonstrated herein, the bacteria of the
genus *Photorhabdus* produce proteins that have functional activity
as defined herein. Of particular interest are proteins produced by
the species *Photorhabdus luminescens*. The inventions herein should
in no way be limited to the strains which are disclosed herein.

20   These strains illustrate for the first time that proteins produced
by diverse isolates of *Photorhabdus* are toxic upon exposure to
insects. Thus, included within the inventions described herein are
the strains specified herein and any mutants thereof, as well as
any strains or species of the genus *Photorhabdus* that have the

25   functional activity described herein.
        There are several terms that are used herein that have a
particular meaning and are as follows:

By "functional activity" it is meant herein that the protein

30   toxin(s) function as insect control agents in that the proteins are
orally active, or have a toxic effect, or are able to disrupt or
deter feeding, which may or may not cause death of the insect.
When an insect comes into contact with an effective amount of toxin
delivered via transgenic plant expression, formulated protein

35   compositions(s), sprayable protein composition(s), a bait matrix or
other delivery system, the results are typically death of the
insect, or the insects do not feed upon the source which makes the
toxins available to the insects.

40   By the use of the term "genetic material" herein, it is meant to
include all genes, nucleic acid, DNA and RNA.

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By "homolog" it is meant an amino acid sequence that is identified as possessing homology to a reference W-14 toxin polypeptide amino acid sequence.

5   By "homology" it is meant an amino acid sequence that has a similarity index of at least 33% and/or an identity index of at least 26% to a reference W-14 toxin polypeptide amino acid sequence, as scored by the GAP algorithm using the B10sum 62 protein scoring matrix (Wisconsin Package Version 9.0, Genetics Computer Group
10   (GCG), Madison, WI).

By "identity" is meant an amino acid sequence that contains an identical residue at a given position, following alignment with a reference W-14 toxin polypeptide amino acid sequence by the GAP
15   algorithm.

The protein toxins discussed herein are typically referred to as "insecticides". By insecticides it is meant herein that the protein toxins have a "functional activity" as further defined
20   herein and are used as insect control agents.

By the use of the term "oligonucleotides" it is meant a macromolecule consisting of a short chain of nucleotides of either RNA or DNA. Such length could be at least one nucleotide, but
25   typically are in the range of about 10 to about 12 nucleotides. The determination of the length of the oligonucleotide is well within the skill of an artisan and should not be a limitation herein. Therefore, oligonucleotides may be less than 10 or greater than 12.
30
−   By the use of the term "Photorhabdus toxin" it is meant any protein produced by a Photorhabdus microorganism strain which has functional activity against insects, where the Photorhabdustoxin could be formulated as a sprayable composition, expressed by a
35   transgenic plant, formulated as a bait matrix, delivered via baculovirus, or delivered by any other applicable host or delivery system.

By the use of the term "toxic" or "toxicity" as used herein it is
40   meant that the toxins produced by Photorhabdus have "functional activity" as defined herein.

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By "truncated peptide" it is meant herein to include any peptide
that is fragment(s) of the peptides observed to have functional
activity.

5   By "substantial sequence homology" is meant either: a DNA fragment
having a nucleotide sequence sufficiently similar to another DNA
fragment to produce a protein having similar biochemical properties;
or a polypeptide having an amino acid sequence sufficiently similar
to another polypeptide to exhibit similar biochemical properties.
10

        Fermentation broths from selected strains reported in Table 20
were used to determine the following: breadth of insecticidal
toxin production by the *Photorhabdus* genus, the insecticidal
spectrum of these toxins, and to provide source material to purify
15   the toxin complexes. The strains characterized herein have been
shown to have oral toxicity against a variety of insect orders.
Such insect orders include but are not limited to *Coleoptera,
Homoptera, Lepidoptera, Diptera, Acarina, Hymenoptera* and
Dictyoptera.
20   As with other bacterial toxins, the rate of mutation of the
bacteria in a population causes many related toxins slightly
different in sequence to exist. Toxins of interest here are those
which produce protein complexes toxic to a variety of insects upon
exposure, as described herein. Preferably, the toxins are active
25   against *Lepidoptera, Coleoptera, Homopotera, Diptera, Hymenoptera,
Dictyoptera* and *Acarina.* The inventions herein are intended to
capture the protein toxins homologous to protein toxins produced by
the strains herein and any derivative strains thereof, as well as
any protein toxins produced by *Photorhabdus.* These homologous
30   proteins may differ in sequence, but do not differ in function from
those toxins described herein. Homologous toxins are meant to
include protein complexes of between 300 kDa to 2,000 kDa and are
comprised of at least two (2) subunits, where a subunit is a
peptide which may or may not be the same as the other subunit.
35   Various protein subunits have been identified and are taught in the
Examples herein. Typically, the protein subunits are between about
18 kDa to about 230 kDa; between about 160 kDa to about 230 kDa;
100 kDa to 160 kDa; about 80 kDa to about 100 kDa; and about 50 kDa
to about 80 kDa.
40   As discussed above, some *Photorhabdus* strains can be isolated
from nematodes. Some nematodes, elongated cylindrical parasitic
worms of the phylum *Nematoda*, have evolved an ability to exploit
insect larvae as a favored growth environment. The insect larvae

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provide a source of food for growing nematodes and an environment
in which to reproduce. One dramatic effect that follows invasion
of larvae by certain nematodes is larval death. Larval death
results from the presence of, in certain nematodes, bacteria that
5   produce an insecticidal toxin which arrests larval growth and
inhibits feeding activity.

Interestingly, it appears that each genus of insect parasitic
nematode hosts a particular species of bacterium, uniquely adapted
for symbiotic growth with that nematode. In the interim since this
10   research was initiated, the name of the bacterial genus *Xenorhabdus*
was reclassified into the *Xenorhabdus* and the *Photorhabdus*.
Bacteria of the genus *Photorhabdus* are characterized as being
symbionts of *Heterorhabditus* nematodes while *Xenorhabdus* species
are symbionts of the *Steinernema* species. This change in
15   nomenclature is reflected in this specification, but in no way
should a change in nomenclature alter the scope of the inventions
described herein.

The peptides and genes that are disclosed herein are named
according to the guidelines recently published in the Journal of
20   Bacteriology "Instructions to Authors" p. *i-xii* (Jan. 1996), which
is incorporated herein by reference. The following peptides and
genes were isolated from *Photorhabdus* strain W-14.

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Table 1
Peptide/Gene Nomenclature
Toxin Complex

1 Peptide Name	2 Peptide Sequence ID No.*	3 Gene Name	4 Gene Sequence ID No.*
tca genomic region			
TcaA	34 [c]	tcaA	33
TcaA$_i$	pro-peptide	tcaA	-
TcaA$_{ii}$	[15][a], 34[c]	tcaA	-
TcaA$_{iii}$	[4][a], 35[c]	tcaA	-
TcaA$_{iv}$	[62][a]	tcaA	-
TcaB	[3][a], (19, 20)[b], 26[c]	tcaB	25
TcaB$_i$	[3][a], (19, 20)[b], 28[c]	tcaB	27
TcaB$_{ii}$	[5][a], 30[c]	tcaB	29
TcaC	[2][a], 32[c]	tcaC	31
tcb genomic region			
TcbA	12[c], [16][a], (21, 22, 23, 24)[b]	tcbA	11
TcbA$_i$	pro-peptide	tcbA	-
TcbA$_{ii}$	[1][a], (21, 22, 23, 24)[b], 53[c]	tcbA	52
TcbA$_{iii}$	[40][a], 55[c]	tcbA	54
tcc genomic region			
TccA	[8][a], 57[c]	tccA	56
TccB	[7][a], 59[c]	tccB	58
TccC	61[c]	tccC	60
tcd genomic region			
TcdA	(17, 18, 37, 38, 39, 42, 43)[b], 47[c]	tcdA	(36)[d], 46
TcdA$_i$	pro-peptide	tcdA	-
TcdA$_{ii}$	[13][a], (17, 18, 37, 38, 39)[b], 49[c]	tcdA	48
TcdA$_{iii}$	[41][a], (42, 43)[b], 51[c]	tcdA	50
TcdB	[14][a]	tcdB	-

[a]Sequence ID No.'s in brackets are peptide N-termini;
[b]Numbers in parentheses are N-termini of internal peptide tryptic
    fragments
[c]deduced from gene sequence
[d]internal gene fragment

The sequences listed above are grouped by genomic region. More
specifically, the *Photorhabdus luminesence* bacteria (W-14) has at
least four distinct genomic regions- tca, tcb, tcc and tcd. As can
be seen in Table 1, peptide products are produced from these
distinct genomic regions. Furthermore, as illustrated in the
Examples, specifically Examples 15 and 21, individual gene products
produced from three genomic regions are associated with insect
activity. There is also considerable homology between these four
genomic regions.

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As is further illustrated in the Examples, the tcbA gene was
expressed in E. coli as two possible biological active protein
fragments (TcbA and TcbA$_{ii/iii}$). The tcdA gene was also expressed
in E. coli. As illustrated in Example 16, when the native

5   unprocessed TcbA toxin was treated with the endogeneous
metalloproteases or insect gut contents containing proteases, the
TcbA protein toxin was processed into smaller subunits that were
less than the size of the native peptides and Southern Corn
Rootworm activity increased. The smaller toxin peptides remained

10   associated as part of a toxin complex. It may be desirable in some
situations to increase activation of the toxin(s) by proteolytic
processing or using truncated peptides. Thus, it may be more
desirable to use truncated peptide(s) in some applications, i.e.,
commercial transgenic plant applications.

15   In addition to the W-14 strain, there are other species within
the Photorhabdus genus that have functional activity which is
differential (specifically see Tables 20 and 36). Even though there
is differential activity, the amino acid sequences in some cases
have substantial sequence homology. Moreover, the molecular probes

20   indicate that some genes contained in the strains are homologous to
the genes contained in the W-14 strain. In fact all of the strains
illustrated herein have one or more homologs of W-14 toxin genes.
The antibody data in Example 26 and the N-terminal sequence data in
Example 25 further support the conclusion that there is homology and

25   identity (based on amino acid sequence) between the protein toxin(s)
produced by these strains. At the molecular level, the W-14 gene
probes indicated that the homologs or the W-14 genes themselves
(Tables 37, 38, and 39) are dispersed throughout the Photorhabdus
genus. Further, it is possible that new toxin genes exist in other

30   strains which are not homologous to W-14, but maintain overall
protein attributes (see specifically Examples 14 and 25).
       Even though there is homology or identity between toxin genes
produced by the Photorhabdus strains, the strains themselves are
quite diverse. Using polymerase chain reaction technology further

35   discussed in Example 22, most of the strains illustrated herein are
quite distinguishable. For example as can be seen in Figs. 5, the
percentage relative similarity of some of the strains, such as HP88
and NC-1, was about 0.8, which indicates that the strains are
similar, while HP88 and Hb was about 0.1, which indicates

40   substantial diversity. Therefore, even though the insect toxin
genes or gene products that the strains produce are the same or
similar, the strains themselves are diverse.

In view of the data further disclosed in the Examples and
discussions herein, it is clear that a new and unique family of
insecticidal protein toxin(s) has been discovered. It has been
further illustrated herein that these toxin(s) widely exist within
5   bacterial strains of the *Photorhabdus* genus. It may also be the
case that these toxin genes widely exist within the family
Enterobacteracaea. Antibodies prepared as described in Example 21
or gene probes prepared as described in Example 25 may be used to
further screen for bacterial strains within the family
10   *Enterobacteracaea* that produce the homologous toxin(s) that have
functional activity. It may also be the case that specific primer
sets exist that could facilitate the identification of new genes
within the *Photorhabdus* genus or family *Enterobacteracaea*.

      As stated above, the antibodies may be used to rapidly screen
15   bacteria of the genus *Photorhabdus* or the family *Enterbacteracaea*
for homologous toxin products as illustrated in Example 26. Those
skilled in the art are quite familiar with the use of antibodies as
an analysis or screening tool (see US Patent No. 5,430,137, which is
incorporated herein by reference). Moreover, it is generally
20   accepted in the literature that antibodies are elicited against 6 to
20 amino acid residue segments that tend to occupy exposed surface
of polypeptides (Current Protocols in Immunology, Coligan et al,
National Institutes of Health, John Wiley & Sons, Inc.). Usually
the amino acid consist of contiguous amino acid residues, however,
25   in certain cases they may be formed by non-contiguous amino acids
that are constrained by specific conformation. The amino acid
segments recognized by antibodies are highly specific and commonly
referred to epitopes. The amino acid fragment can be generated by
chemical and/or enzymatic cleavage of the native protein, by
30   automated, solid-phase peptide synthesis, or by production from
genetic engineering organisms. Polypeptide fragments can be
isolated by a variety and/or combination of HPLC and FPLC
chromatographic methods known in the art. Selection of polypeptide
fragment can be aided by the use of algorithms, for example Kyte and
35   Doolittle, 1982, Journal of Molecular Biology 157: 105-132 and Chou
and Fasman, 1974, Biochemistry 13: 222-245, that predict those
sequences most likely to exposed on the surface of the protein. For
preparation of immunogen containing the polypeptide fragment of
interest, in general, polypeptides are covalently coupled using
40   chemical reactions to carrier proteins such as keyhole limpet
hemocyanin via free amino (lysine), sulfhydyl (cysteine), phenolic
(tyrosine) or carboxylic (aspartate or glutamate) groups. Immunogen
with an adjuvant is injected in animals, such as mice or rabbits, or

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chickens to elicit an immune response against the immunogen.
Analysis of antibody titer in antisera of inject animals against
polypeptide fragment can be determined by a variety of immunological
methods such as ELISA and Western blot. Alternatively, monoclonal
5   antibodies can be prepared using spleen cells of the injected animal
for fusion with tumor cells to produce immortalized hybridomas cells
producing a single antibody species. Hybridomas cells are screened
using immunological methods to select lines that produce a specific
antibody to the polypeptide fragment of interest. Purification of
10   antibodies from different sources can be performed by a variety of
antigen affinity or antibody affinity columns or other
chromatographic HPLC or FPLC methods.

        The toxins described herein are quite unique in that the
toxins have functional activity, which is key to developing an
15   insect management strategy. In developing an insect management
strategy, it is possible to delay or circumvent the protein
degradation process by injecting a protein directly into an
organism, avoiding its digestive tract. In such cases, the protein
administered to the organism will retain its function until it is
20   denatured, non-specifically degraded, or eliminated by the immune
system in higher organisms. Injection into insects of an
insecticidal toxin has potential application only in the
laboratory, and then only on large insects which are easily
injected. The observation that the insecticidal protein toxins
25   herein described exhibits their toxic activity after oral ingestion
or contact with the toxins permits the development of an insect
management plan based solely on the ability to incorporate the
protein toxins into the insect diet. Such a plan could result in
the production of insect baits.

30   The *Photorhabdus* toxins may be administered to insects in a
purified form. The toxins may also be delivered in amounts from
about 1 to about 100 mg / liter of broth. This may vary upon
formulation condition, conditions of the inoculum source,
techniques for isolation of the toxin, and the like. The toxins
35   may be administered as an exudate secretion or cellular protein
originally expressed in a heterologous prokaryotic or eukaryotic
host. Bacteria are typically the hosts in which proteins are
expressed. Eukaryotic hosts could include but are not limited to
plants, insects and yeast. Alternatively, the toxins may be
40   produced in bacteria or transgenic plants in the field or in the
insect by a baculovirus vector. Typically the toxins will be
introduced to the insect by incorporating one or more of the toxins
into the insects' feed.

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Complete lethality to feeding insects is useful but is not required to achieve useful toxicity. If the insects avoid the toxin or cease feeding, that avoidance will be useful in some applications, even if the effects are sublethal. For example, if

5   insect resistant transgenic crop plants are desired, a reluctance of insects to feed on the plants is as useful as lethal toxicity to the insects since the ultimate objective is protection of the plants rather than killing the insect.

There are many other ways in which toxins can be incorporated

10 into an insect's diet. As an example, it is possible to adulterate the larval food source with the toxic protein by spraying the food with a protein solution, as disclosed herein. Alternatively, the purified protein could be genetically engineered into an otherwise harmless bacterium, which could then be grown in culture, and

15 either applied to the food source or allowed to reside in the soil in an area in which insect eradication was desirable. Also, the protein could be genetically engineered directly into an insect food source. For instance, the major food source of many insect larvae is plant material.

20 By incorporating genetic material that encodes the insecticidal properties of the *Photorhabdus* toxins into the genome of a plant eaten by a particular insect pest, the adult or larvae would die after consuming the food plant. Numerous members of the monocotyledonous and dictyledenous genera have been transformed.

25 Transgenic agronmonic crops as well as fruits and vegetables are of commercial interest. Such crops include but are not limited to maize, rice, soybeans, canola, sunflower, alfalfa, sorghum, wheat, cotton, peanuts, tomatoes, potatoes, and the like. Several techniques exist for introducing foreign genetic material into

30 plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (U.S. Patents 4,945,050 to Cornell and 5,141,131 to DowElanco). Plants may be transformed using *Agrobacterium* technology, see U.S.

35 Patent 5,177,010 to University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, U.S. Patents 5,149,645, 5,469,976, 5,464,763 and 4,940,838 and 4,693,976 to Schilperoot, European Patent Applications 116718, 290799, 320500

40 all to MaxPlanck, European Patent Applications 604662 and 627752 to Japan Tobacco, European Patent Applications 0267159, and 0292435 and U.S. Patent 5,231,019 all to Ciba Geigy, U.S. Patents 5,463,174 and 4,762,785 both to Calgene, and U.S. Patents 5,004,863 and

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5,159,135 both to Agracetus. Other transformation technology
includes whiskers technology, see U.S. Patents 5,302,523 and
5,464,765 both to Zeneca. Electroporation technology has also been
used to transform plants, see WO 87/06614 to Boyce Thompson
5   Institute, 5,472,869 and 5,384,253 both to Dekalb, WO9209696 and
WO9321335 both to PGS. All of these transformation patents and
publications are incorporated by reference. In addition to
numerous technologies for transforming plants, the type of tissue
which is contacted with the foreign genes may vary as well. Such
10 tissue would include but would not be limited to embryogenic
tissue, callus tissue type I and II, hypocotyl, meristem, and the
like. Almost all plant tissues may be transformed during
dedifferentiation using appropriate techniques within the skill of
an artisan.
15   Another variable is the choice of a selectable marker. The
preference for a particular marker is at the discretion of the
artisan, but any of the following selectable markers may be used
along with any other gene not listed herein which could function as
a selectable marker. Such selectable markers include but are not
20 limited to aminoglycoside phosphotransferase gene of transposon Tn5
(Aph II) which encodes resistance to the antibiotics kanamycin,
neomycin and G418, as well as those genes which code for resistance
or tolerance to glyphosate; hygromycin; methotrexate;
phosphinothricin (bialophos); imidazolinones, sulfonylureas and
25 triazolopyrimidine herbicides, such as chlorosulfuron; bromoxynil,
dalapon and the like.
        In addition to a selectable marker, it may be desirous to use
a reporter gene. In some instances a reporter gene may be used
without a selectable marker. Reporter genes are genes which are
30 typically not present or expressed in the recipient organism or
tissue. The reporter gene typically encodes for a protein which
provides for some phenotypic change or enzymatic property.
Examples of such genes are provided in K. Weising et al. Ann. Rev.
Genetics, 22, 421 (1988), which is incorporated herein by
35 reference. A preferred reporter gene is the glucuronidase (GUS)
gene.
        Regardless of transformation technique, the gene is preferably
incorporated into a gene transfer vector adapted to express the
Photorhabdus toxins in the plant cell by including in the vector a
40 plant promoter. In addition to plant promoters, promoters from a
variety of sources can be used efficiently in plant cells to
express foreign genes. For example, promoters of bacterial origin,
such as the octopine synthase promoter, the nopaline synthase

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promoter, the mannopine synthase promoter; promoters of viral origin, such as the cauliflower mosaic virus (35S and 19S), reengineered 35S, known as 35T (see PCT/US96/16582, WO 97/13402 published April 17, 1997, which is incorporated herein by

5   reference) and the like may be used. Plant promoters include, but are not limited to ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin promoter, phaseolin promoter, ADH promoter, heat-shock promoters and tissue specific promoters. Promoters may also contain certain enhancer sequence elements that

10   may improve the transcription efficiency. Typical enhancers include but are not limited to Adh-intron 1 and Adh-intron 6. Constitutive promoters may be used. Constitutive promoters direct continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S). Tissue specific promoters are

15   responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP) and these promoters may also be used. Promoters may also be are active during a certain stage of the plants' development as well as active in plant tissues and organs. Examples of such promoters include

20   but are not limited to pollen-specific, embryo specific, corn silk specific, cotton fiber specific, root specific, seed endosperm specific promoters and the like.

          Under certain circumstances it may be desirable to use an inducible promoter. An inducible promoter is responsible for

25   expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; and stress. Other desirable transcription and translation elements that function in plants may be used. Numerous plant-specific gene transfer vectors are known

30   to the art.

          In addition, it is known that to obtain high expression of bacterial genes in plants it is preferred to reengineer the bacterial genes so that they are more efficiently expressed in the cytoplasm of plants. Maize is one such plant where it is preferred

35   to reengineer the bacterial gene(s) prior to transformation to increase the expression level of the toxin in the plant. One reason for the reengineering is the very low G+C content of the native bacterial gene(s) (and consequent skewing towards high A+T content). This results in the generation of sequences mimicking or

40   duplicating plant gene control sequences that are known to be highly A+T rich. The presence of some A+T-rich sequences within the DNA of the gene(s) introduced into plants (e.g., TATA box regions normally found in gene promoters) may result in aberrant

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transcription of the gene(s). On the other hand, the presence of other regulatory sequences residing in the transcribed mRNA (e.g., polyadenylation signal sequences (AAUAAA), or sequences complementary to small nuclear RNAs involved in pre-mRNA splicing)
5   may lead to RNA instability. Therefore, one goal in the design of reengineered bacterial gene(s), more preferably referred to as plant optimized gene(s), is to generate a DNA sequence having a higher G+C content, and preferably one close to that of plant genes coding for metabolic enzymes. Another goal in the design of the
10 plant optimized gene(s) is to generate a DNA sequence that not only has a higher G+C content, but by modifying the sequence changes, should be made so as to not hinder translation.

An example of a plant that has a high G+C content is maize. The table below illustrates how high the G+C content is in maize.
15 As in maize, it is thought that G+C content in other plants is also high.

Table 2
Compilation of G+C Contents of Protein Coding Regions
of Maize Genes

20

Protein Class[a]	Range %G+C	Mean %G+C[b]
Metabolic Enzymes (40)	44.4-75.3	59.0 (8.0)
Storage Proteins		
Group I (23)	46.0-51.9	48.1 (1.3)
Group II (13)	60.4-74.3	67.5 (3.2)
Group I + II (36)	46.0-74.3	55.1 (9.6)[c]
Structural Proteins (18)	48.6-70.5	63.6 (6.7)
Regulatory Proteins (5)	57.2-68.9	62.0 (4.9)
Uncharacterized Proteins (9)	41.5-70.3	64.3 (7.2)
All Proteins (108)	44.4-75.3	60.8 (5.2)

[a]   Number of genes in class given in parentheses.
[b]   Standard deviations given in parentheses.
[c]   Combined groups mean ignored in calculation of
25   overall mean.

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For the data in Table 2, coding regions of the genes were extracted from GenBank (Release 71) entries, and base compositions were calculated using the MacVector™ program (IBI, New Haven, CT). Intron sequences were ignored in the calculations. Group I and II
5   storage protein gene sequences were distinguished by their marked difference in base composition.

Due to the plasticity afforded by the redundancy of the genetic code (i.e., some amino acids are specified by more than one codon), evolution of the genomes of different organisms or classes
10   or organisms has resulted in differential usage of redundant codons. This "codon bias" is reflected in the mean base composition of protein coding regions. For example, organisms with relatively low G+C contents utilize codons having A or T in the third position of redundant codons, whereas those having higher G+C contents
15   utilize codons having G or C in the third position. It is thought that the presence of "minor" codons within a gene's mRNA may reduce the absolute translation rate of that mRNA, especially when the relative abundance of the charged tRNA corresponding to the minor codon is low. An extension of this is that the diminution of
20   translation rate by individual minor codons would be at least additive for multiple minor codons. Therefore, mRNAs having high relative contents of minor codons would have correspondingly low translation rates. This rate would be reflected by the synthesis of low levels of the encoded protein.
25   In order to reengineer the bacterial gene(s), the codon bias of the plant is determined. The codon bias is the statistical codon distribution that the plant uses for coding its proteins. After determining the bias, the percent frequency of the codons in the gene(s) of interest is determined. The primary codons
30   preferred by the plant should be determined as well as the second and third choice of preferred codons. The amino acid sequence of the protein of interest is reverse translated so that the resulting nucleic acid sequence codes for the same protein as the native bacterial gene, but the resulting nucleic acid sequence corresponds
35   to the first preferred codons of the desired plant. The new sequence is analyzed for restriction enzyme sites that might have been created by the modification. The identified sites are further modified by replacing the codons with second or third choice preferred codons. Other sites in the sequence which could affect
40   the transcription or translation of the gene of interest are the exon:intron 5' or 3' junctions, poly A addition signals, or RNA polymerase termination signals. The sequence is further analyzed and modified to reduce the frequency of TA or GC doublets. In

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addition to the doublets, G or C sequence blocks that have more than about four residues that are the same can affect transcription of the sequence. Therefore, these blocks are also modified by replacing the codons of first or second choice, etc. with the next

5   preferred codon of choice. It is preferred that the plant optimized gene(s) contains about 63% of first choice codons, between about 22% to about 37% second choice codons, and between 15% and 0% third choice codons, wherein the total percentage is 100%. Most preferred the plant optimized gene(s) contain about 63%

10   of first choice codons, at least about 22% second choice codons, about 7.5% third choice codons, and about 7.5% fourth choice codons, wherein the total percentage is 100%. The method described above enables one skilled in the art to modify gene(s) that are foreign to a particular plant so that the genes are

15   optimally expressed in plants. The method is further illustrated in application PCT/US96/16582, WO 97/13402 published April 17, 1997.

Thus, in order to design plant optimized gene(s) the amino acid sequence of the toxins are reverse translated into a DNA

20   sequence, utilizing a nonredundant genetic code established from a codon bias table compiled for the gene DNA sequence for the particular plant being transformed. The resulting DNA sequence, which is completely homogeneous in codon usage, is further modified to establish a DNA sequence that, besides having a higher degree of

25   codon diversity, also contains strategically placed restriction enzyme recognition sites, desirable base composition, and a lack of sequences that might interfere with transcription of the gene, or translation of the product mRNA.

It is theorized that bacterial genes may be more easily

30   expressed in plants if the bacterial genes are expressed in the plastids. Thus, it may be possible to express bacterial genes in plants, without optimizing the genes for plant expression, and obtain high express of the protein. See U.S. Patent Nos. 4,762,785; 5,451,513 and 5,545,817, which are incorporated herein

35   by reference.

One of the issues regarding commercial exploiting transgenic plants is resistance management. This is of particular concern with *Bacillus thuringiensis* toxins. There are numerous companies commerically exploiting *Bacillus thuringiensis* and there has been

40   much concern about *Bt* toxins becoming resistant. One strataegy for insect resistant management would be to combine the toxins produced by *Photorhabdus* with toxins such as *Bt*, vegetative insect proteins (Ciba Geigy) or other toxins. The combinations could be formulated

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for a sprayable application or could be molecular combinations.
Plants could be transformed with *Photorhabdus* genes that produce
insect toxins and other insect toxin genes such as *Bt* as with other
insect toxin genes such as *Bt*.

5   European Patent Application 0400246A1 describes transformation
of 2 *Bt* in a plant, which could be any 2 genes. Another way to
produce a transgenic plant that contains more than one insect
resistant gene would be to produce two plants, with each plant
containing an insect resistant gene. These plants would be
10   backcrossed using traditional plant breeding techniques to produce
a plant containing more than one insect resistant gene.

          In addition to producing a transformed plant containing plant
optimized gene(s), there are other delivery systems where it may be
desirable to reengineer the bacterial gene(s). Along the same
15   lines, a genetically engineered, easily isolated protein toxin
fusing together both a molecule attractive to insects as a food
source and the insecticidal activity of the toxin may be engineered
and expressed in bacteria or in eukaryotic cells using standard,
well-known techniques. After purification in the laboratory such a
20   toxic agent with "built-in" bait could be packaged inside standard
insect trap housings.

          Another delivery scheme is the incorporation of the genetic
material of toxins into a baculovirus vector. Baculoviruses infect
particular insect hosts, including those desirably targeted with
25   the *Photorhabdus* toxins. Infectious baculovirus harboring an
expression construct for the *Photorhabdus* toxins could be
introduced into areas of insect infestation to thereby intoxicate
or poison infected insects.

          Transfer of the insecticidal properties requires nucleic acid
30   sequences encoding the coding the amino acid sequences for the
Photorhabdus toxins integrated into a protein expression vector
appropriate to the host in which the vector will reside. One way
to obtain a nucleic acid sequence encoding a protein with
insecticidal properties is to isolate the native genetic material
35   which produces the toxins from *Photorhabdus*, using information
deduced from the toxin's amino acid sequence, large portions of
which are set forth below. As described below, methods of
purifying the proteins responsible for toxin activity are also
disclosed.

40   Using N-terminal amino acid sequence data, such as set forth
below, one can construct oligonucleotides complementary to all, or
a section of, the DNA bases that encode the first amino acids of
the toxin. These oligonucleotides can be radiolabeled and used as

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molecular probes to isolate the genetic material from a genomic
genetic library built from genetic material isolated from strains
of *Photorhabdus*. The genetic library can be cloned in plasmid,
cosmid, phage or phagemid vectors. The library could be
5   transformed into *Escherichia coli* and screened for toxin production
by the transformed cells using antibodies raised against the toxin
or direct assays for insect toxicity.

   This approach requires the production of a battery of
oligonucleotides, since the degenerate genetic code allows an amino
10 acid to be encoded in the DNA by any of several three-nucleotide
combinations. For example, the amino acid arginine can be encoded
by nucleic acid triplets CGA, CGC, CGG, CGT, AGA, and AGG. Since
one cannot predict which triplet is used at those positions in the
toxin gene, one must prepare oligonucleotides with each potential
15 triplet represented. More than one DNA molecule corresponding to a
protein subunit may be necessary to construct a sufficient number
of oligonucleotide probes to recover all of the protein subunits
necessary to achieve oral toxicity.

   From the amino acid sequence of the purified protein, genetic
20 materials responsible for the production of toxins can readily be
isolated and cloned, in whole or in part, into an expression vector
using any of several techniques well-known to one skilled in the
art of molecular biology. A typical expression vector is a DNA
plasmid, though other transfer means including, but not limited to,
25 cosmids, phagemids and phage are also envisioned. In addition to
features required or desired for plasmid replication, such as an
origin of replication and antibiotic resistance or other form of a
selectable marker such as the *bar* gene of *Streptomyces*
hygroscopicus or *viridochromogenes*, protein expression vectors
30 normally additionally require an expression cassette which
incorporates the cis-acting sequences necessary for transcription
and translation of the gene of interest. The cis-acting sequences
required for expression in prokaryotes differ from those required
in eukaryotes and plants.
35   A eukaryotic expression cassette requires a transcriptional
promoter upstream (5') to the gene of interest, a transcriptional
termination region such as a poly-A addition site, and a ribosome
binding site upstream of the gene of interest's first codon. In
bacterial cells, a useful transcriptional promoter that could be
40 included in the vector is the T7 RNA Polymerase-binding promoter.
Promoters, as previously described herein, are known to efficiently
promote transcription of mRNA. Also upstream from the gene of
interest the vector may include a nucleotide sequence encoding a

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signal sequence known to direct a covalently linked protein to a
particular compartment of the host cells such as the cell surface.

    Insect viruses, or baculoviruses, are known to infect and
adversely affect certain insects. The affect of the viruses on
5   insects is slow, and viruses do not stop the feeding of insects.
Thus viruses are not viewed as being useful as insect pest control
agents. Combining the *Photorhabdus* toxins genes into a baculovirus
vector could provide an efficient way of transmitting the toxins
while increasing the lethality of the virus. In addition, since
10 different baculoviruses are specific to different insects, it may
be possible to use a particular toxin to selectively target
particularly damaging insect pests. A particularly useful vector
for the toxins genes is the nuclear polyhedrosis virus. Transfer
vectors using this virus have been described and are now the
15 vectors of choice for transferring foreign genes into insects. The
virus-toxin gene recombinant may be constructed in an orally
transmissible form. Baculoviruses normally infect insect victims
through the mid-gut intestinal mucosa. The toxin gene inserted
behind a strong viral coat protein promoter would be expressed and
20 should rapidly kill the infected insect.

    In addition to an insect virus or baculovirus or transgenic
plant delivery system for the protein toxins of the present
invention, the proteins may be encapsulated using *Bacillus
thuringiensis* encapsulation technology such as but not limited to
25 U.S. Patent Nos. 4,695,455; 4,695,462; 4,861,595 which are all
incorporated herein by reference. Another delivery system for the
protein toxins of the present invention is formulation of the
protein into a bait matrix, which could then be used in above and
below ground insect bait stations. Examples of such technology
30 include but are not limited to PCT Patent Application WO 93/23998,
which is incorporated herein by reference.

    As is described above, it might become necessary to modify the
sequence encoding the protein when expressing it in a non-native
host, since the codon preferences of other hosts may differ from
35 that of *Photorhabdus*. In such a case, translation may be quite
inefficient in a new host unless compensating modifications to the
coding sequence are made. Additionally, modifications to the amino
acid sequence might be desirable to avoid inhibitory cross-
reactivity with proteins of the new host, or to refine the
40 insecticidal properties of the protein in the new host. A
genetically modified toxin gene might encode a toxin exhibiting,
for example, enhanced or reduced toxicity, altered insect

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resistance development, altered stability, or modified target
species specificity.

In addition to the *Photorhabdus* genes encoding the toxins, the
scope of the present invention is intended to include related
5   nucleic acid sequences which encode amino acid biopolymers
homologous to the toxin proteins and which retain the toxic effect
of the *Photorhabdus* proteins in insect species after oral
ingestion.

For instance, the toxins used in the present invention seem to
10   first inhibit larval feeding before death ensues. By manipulating
the nucleic acid sequence of *Photorhabdus* toxins or its controlling
sequences, genetic engineers placing the toxin gene into plants
could modulate its potency or its mode of action to, for example,
keep the eating-inhibitory activity while eliminating the absolute
15   toxicity to the larvae. This change could permit the transformed
plant to survive until harvest without having the unnecessarily
dramatic effect on the ecosystem of wiping out all target insects.
All such modifications of the gene encoding the toxin, or of the
protein encoded by the gene, are envisioned to fall within the
20   scope of the present invention.

Other envisioned modifications of the nucleic acid include the
addition of targeting sequences to direct the toxin to particular
parts of the insect larvae for improving its efficiency.

Strains W-14, ATCC 55397, 43948, 43949, 43950, 43951, 43952
25   have been deposited in the American Type Culture Collection, 12301
Parklawn Drive, Rockville, MD 20852 USA. Amino acid and nucleotide
sequence data for the W-14 native toxin (ATCC 55397) is presented
below. Isolation of the genomic DNA for the toxins from the
bacterial hosts is also exemplified herein. The other strains
30   identified herein have been deposited with the United States
Department of Agriculture, 1815 North University Drive, Peoria, IL
61604.

Standard and molecular biology techniques were followed and
taught in the specification herein. Additional information may be
35   found in Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989),
<u>Molecular Cloning, A Laboratory Manual</u>, Cold Spring Harbor Press;
<u>Current Protocalsin Molecular Biology</u>, ed. F. M. Ausubel et al.,
(1997), which are both incorporated herein by reference.

40   The following abbreviations are used throughout the Examples: Tris
= tris (hydroxymethyl) amino methane; SDS = sodium dodecyl sulfate;
EDTA = ethylenediaminetetraacetic acid, IPTG = isopropylthio-B-
galactoside, X-gal = 5-bromo-4-chloro-3-indoyl-B-D-galactoside,

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CTAB = cetyltrimethylammonium bromide; kbp = kilobase pairs; dATP, dCTP, dGTP, dTTP, I = 2'-deoxynucleoside 5'-triphosphates of adenine, cytosine, guanine, thymine, and inosine, respectively; ATP = adenosine 5' triphosphate.

5

Example 1
Purification of Toxin from *Photorhabdus luminescens* and
Demonstration of Toxicity after Oral Delivery of Purified Toxin

10   The insecticidal protein toxin of the present invention was purified from *Photorhabdus luminescens* strain W-14, ATCC Accession Number 55397. Stock cultures of *Photorhabdus luminescens* were maintained on petri dishes containing 2% Proteose Peptone No. 3 (i.e., PP3, Difco Laboratories, Detroit MI) in 1.5% agar, incubated
15 at 25°C and transferred weekly. Colonies of the primary form of the bacteria were inoculated into 200 ml of PP3 broth supplemented with 0.5% polyoxyethylene sorbitan mono-stearate (Tween 60, Sigma Chemical Company, St. Louis, MO) in a one liter flask. The broth cultures were grown for 72 hours at 30°C on a rotary shaker. The
20 toxin proteins can be recovered from cultures grown in the presence or absence of Tween; however, the absence of Tween can affect the form of the bacteria grown and the profile of proteins produced by the bacteria. In the absence of Tween, a variant shift occurs insofar as the molecular weight of at least one identified toxin
25 subunit shifts from about 200 kDa to about 185 kDa.

The 72 hour cultures were centrifuged at 10,000 x g for 30 minutes to remove cells and debris. The supernatant fraction that contained the insecticidal activity was decanted and brought to 50 mM K_2HPO_4 by adding an appropriate volume of 1.0 M K_2HPO_4. The pH
30 was adjusted to 8.6 by adding potassium hydroxide. This supernatant fraction was then mixed with DEAE-Sephacel (Pharmacia LKB Biotechnology) which had been equilibrated with 50 mM K_2HPO_4. The toxic activity was adsorbed to the DEAE resin. This mixture was then poured into a 2.6 x 40 cm column and washed with 50 mM
35 K_2HPO_4 at room temperature at a flow rate of 30 ml/hr until the effluent reached a steady baseline UV absorbance at 280 nm. The column was then washed with 150 mM KCl until the effluent again reached a steady 280 nm baseline. Finally the column was washed with 300 mM KCl and fractions were collected.
40   Fractions containing the toxin were pooled and filter sterilized using a 0.2 micron pore membrane filter. The toxin was then concentrated and equilibrated to 100 mM KPO_4, pH 6.9, using an ultrafiltration membrane with a molecular weight cutoff of 100 kDa

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at 4°C (Centriprep 100, Amicon Division-W.R. Grace and Company). A
3 ml sample of the toxin concentrate was applied to the top of a
2.6 x 95 cm Sephacryl S-400 HR gel filtration column (Pharmacia LKB
Biotechnology). The eluent buffer was 100 mM KPO₄, pH 6.9, which

5   was run at a flow rate of 17 ml/hr, at 4°C. The effluent was
monitored at 280 nm.

    Fractions were collected and tested for toxic activity.
Toxicity of chromatographic fractions was examined in a biological
assay using *Manduca sexta* larvae. Fractions were either applied

10 directly onto the insect diet (Gypsy moth wheat germ diet, ICN
Biochemicals Division - ICN Biomedicals, Inc.) or administered by
intrahemocelic injection of a 5 µl sample through the first proleg
of 4th or 5th instar larva using a 30 gauge needle. The weight of
each larva within a treatment group was recorded at 24 hour

15 intervals. Toxicity was presumed if the insect ceased feeding and
died within several days of consuming treated insect diet or if
death occurred within 24 hours after injection of a fraction.

    The toxic fractions were pooled and concentrated using the
Centriprep-100 and were then analyzed by HPLC using a 7.5 mm x 60

20 cm TSK-GEL G-4000 SW gel permeation column with 100 mM potassium
phosphate, pH 6.9 eluent buffer running at 0.4 ml/min. This
analysis revealed the toxin protein to be contained within a single
sharp peak that eluted from the column with a retention time of
approximately 33.6 minutes. This retention time corresponded to an

25 estimated molecular weight of 1,000 kDa. Peak fractions were
collected for further purification while fractions not containing
this protein were discarded. The peak eluted from the HPLC absorbs
UV light at 218 and 280 nm but did not absorb at 405 nm.
Absorbance at 405 nm was shown to be an attribute of xenorhabdin

30 antibiotic compounds.

    Electrophoresis of the pooled peak fractions in a non-
denaturing agarose gel (Metaphor Agarose, FMC BioProducts) showed
that two protein complexes are present in the peak. The peak
material, buffered in 50 mM Tris-HCl, pH 7.0, was separated on a

35 1.5% agarose stacking gel buffered with 100 mM Tris-HCl at pH 7.0
and 1.9% agarose resolving gel buffered with 200 mM Tris-borate at
pH 8.3 under standard buffer conditions (anode buffer 1M Tris-HCl,
pH 8.3; cathode buffer 0.025 M Tris, 0.192 M glycine). The gels
were run at 13 mA constant current at 15°C until the phenol red

40 tracking dye reached the end of the gel. Two protein bands were
visualized in the agarose gels using Coomassie brilliant blue
staining.

The slower migrating band was referred to as "protein band 1"
and faster migrating band was referred to as "protein band 2." The
two protein bands were present in approximately equal amounts. The
Coomassie stained agarose gels were used as a guide to precisely
5   excise the two protein bands from unstained portions of the gels.
The excised pieces containing the protein bands were macerated and
a small amount of sterile water was added. As a control, a portion
of the gel that contained no protein was also excised and treated
in the same manner as the gel pieces containing the protein.
10   Protein was recovered from the gel pieces by electroelution into
100 mM Tris-borate pH 8.3, at 100 volts (constant voltage) for two
hours. Alternatively, protein was passively eluted from the gel
pieces by adding an equal volume of 50 mM Tris-HCl, pH 7.0, to the
gel pieces, then incubating at 30°C for 16 hours. This allowed the
15   protein to diffuse from the gel into the buffer, which was then
collected.

        Results of insect toxicity tests using HPLC-purified toxin
(33.6 min. peak) and agarose gel purified toxin demonstrated
toxicity of the extracts. Injection of 1.5 µg of the HPLC purified
20   protein kills within 24 hours. Both protein bands 1 and 2,
recovered from agarose gels by passive elution or electroelution,
were lethal upon injection. The protein concentration estimated
for these samples was less than 50 ng/larva. A comparison of the
weight gain and the mortality between the groups of larvae injected
25   with protein bands 1 or 2 indicate that protein band 1 was more
toxic by injection delivery.

        When HPLC-purified toxin was applied to larval diet at a
concentration of 7.5 µg/larva, it caused a halt in larval weight
gain (24 larvae tested). The larvae begin to feed, but after
30   consuming only a very small portion of the toxin treated diet they
began to show pathological symptoms induced by the toxin and the
larvae cease feeding. The insect frass became discolored and most
larva showed signs of diarrhea. Significant insect mortality
resulted when several 5 µg toxin doses were applied to the diet
35   over a 7-10 day period.

        Agarose-separated protein band 1 significantly inhibited
larval weight gain at a dose of 200 ng/larva. Larvae fed similar
concentrations of protein band 2 were not inhibited and gained
weight at the same rate as the control larvae. Twelve larvae_were
40   fed eluted protein and 45 larvae were fed protein-containing
agarose pieces. These two sets of data indicate that protein band
1 was orally toxic to Manduca sexta. In this experiment it
appeared that protein band 2 was not toxic to Manduca sexta.

Further analysis of protein bands 1 and 2 by SDS-PAGE under
denaturing conditions showed that each band was composed of several
smaller protein subunits. Proteins were visualized by Coomassie
brilliant blue staining followed by silver staining to achieve
5   maximum sensitivity.

The protein subunits in the two bands were very similar.
Protein band 1 contains 8 protein subunits of 25.1, 56.2, 60.8,
65.6, 166, 171, 184 and 208 kDa. Protein band 2 had an identical
profile except that the 25.1, 60.8, and 65.6 kDa proteins were not
10   present. The 56.2, 60.8, 65.6, and 184 kDa proteins were present
in the complex of protein band 1 at approximately equal
concentrations and represent 80% or more of the total protein
content of that complex.

The native HPLC-purified toxin was further characterized as
15   follows. The toxin was heat labile in that after being heated to
60°C for 15 minutes it lost its ability to kill or to inhibit
weight gain when injected or fed to *Manduca sexta* larvae. Assays
were designed to detect lipase, type C phospholipase, nuclease or
red blood cell hemolysis activities and were performed with
20   purified toxin. None of these activities were present. Antibiotic
zone inhibition assays were also done and the purified toxin failed
to inhibit growth of Gram-negative or -positive bacteria, yeast or
filamentous fungi, indicating that the toxic is not a xenorhabdin
antibiotic.

25   The native HPLC-purified toxin was tested for ability to kill
insects other than *Manduca sexta*. Table 3 lists insects killed by
the HPLC-purified *Photorhabdus luminescens* toxin in this study.

<div align="center">

Table 3

30   Insects Killed by *Photorhabdus luminescens* Toxin

</div>

Common Name	Order	Genus and species	Route of Delivery
Tobacco horn worm	Lepidoptera	*Manduca sexta*	Oral and injected
Mealworm	Coleoptera	*Tenebrio molitor*	Oral
Pharaoh ant	Hymenoptera	*Monomorium pharoanis*	Oral
German cockroach	Dictyoptera	*Blattella germanica*	Oral and injected
Mosquito	Diptera	*Aedes aegypti*	Oral

<u>Further Characterization of the High Molecular Weight Toxin Complex</u>

        In yet further analysis, the toxin protein complex was
subjected to further characterization from W-14 growth medium. The
culture conditions and initial purification steps through the S-400
5   HR column were identical to those described above. After isolation
of the high molecular weight toxin complex from the S-400 HR column
fractions, the toxic fractions were equilibrated with 10 mM Tris-
HCl, pH 8.6, and concentrated in the centriplus 100 (Amicon)
concentrators. The protein toxin complex was then applied to a weak
10 anion exchange (WAX) column, Vydac 301VPH575 (Hesparia, CA), at a
flow rate of 0.5 ml/min. The proteins were eluted with a linear
potassium chloride gradient, 0-250 mM KCl, in 10 mM Tris-HCl pH 8.6
for 50 min. Eight protein peaks were detected by absorbance at 280
nm.

15   Bioassays using neonate southern corn rootworm (*Diabrotica
undecimpunctata howardi*, SCR) larvae and tobacco horn worm (*Manduca
sexta*, THW) were performed on all fractions eluted from the HPLC
column. THW were grown on Gypsy Moth wheat germ diet (ICN) at 25°C
with a 16 hr light 8 hr dark cycle. SCR were grown on Southern Corn
20 Rootworm Larval Insecta-Diet (BioServ) at 25°C with a 16 hr light /
8 hr dark cycle.

        The highest mortality for SCR and THW larvae was observed for
peak 6, which eluted with ca. 112 mM to 132mM KCl. SDS-PAGE
analysis of peak 6 showed predominant peptides of 170 kDa, 66 kDa,
25 63 kDa, 59.5 kDa and 31 kDa. Western blot analysis was performed on
peak 6 protein fraction with a mixture of polyclonal antibodies made
against TcaA$_{ii}$-syn, TcaA$_{iii}$-syn, TcaB$_{ii}$-syn, TcaC-syn, and TcbA$_{ii}$-syn
peptides (described in Example 21) and C5F2, a monoclonal antibody
against the TcbA$_{iii}$ peptide. Peak 6 contained immuno-reactive bands
30 of 170 kDa, 90 kDa, 66 kDa, 59.5 kDa and 31 kDa. These are very
close to the predicted sizes for the TcaC (166 kDa), TcaA$_{ii}$+ TcaA$_{iii}$

(92 kDa), TcaA$_{iii}$ (66 kDa), TcaB$_{ii}$ (60 kDa) and TcaA$_{ii}$ (25 kDa),
respectively. Peak 6 which was further analyzed by native agarose
gel electrophoresis, as described herein, migrated as a single band
35 with similar mobility to that of band 1.

        The protein concentration of the purified peak 6 toxin protein
was determined using the BCA reagents (Pierce). Dilutions of the
protein were made in 10 mM Tris, pH 8.6 and applied to the diet
bioassays. After 240 hours all neonate larvae on diet bioassays
40 that received 450 ng or greater of the peak 6 protein fraction were
dead. The group of larvae that received 90 ng of the same fraction

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had 40% mortality. After 240 hrs the survivors that received 90 ng
and 20 ng of peak 6 protein fraction were ca. 10% and 70%,
respectively, of the control weight.

5   Example 2
                         Insecticide Utility

        The *Photorhabdus luminescens* utility and toxicity were further
    characterized. *Photorhabdus luminescens* (strain W-14) culture
10 broth was produced as follows. The production medium was 2% Bacto
    Proteose Peptone® Number 3 (PP3, Difco Laboratories, Detroit,
    Michigan) in Milli-Q® deionized water. Seed culture flasks
    consisted of 175 ml medium placed in a 500 ml tribaffled flask with
    a Delong neck, covered with a Kaput and autoclaved for 20 minutes,
15 T=250°F. Production flasks consisted of 500 mls in a 2.8 liter 500
    ml tribaffled flask with a Delong neck, covered by a Shin-etsu
    silicon foam closure. These were autoclaved for 45 minutes,
    T=250°F. The seed culture was incubated at 28°C at 150 rpm in a
    gyrotory shaking incubator with a 2 inch throw. After 16 hours of
20 growth, 1% of the seed culture was placed in the production flask
    which was allowed to grow for 24 hours before harvest. Production
    of the toxin appears to be during log phase growth. The microbial
    broth was transferred to a 1L centrifuge bottle and the cellular
    biomass was pelleted (30 minutes at 2500 RPM at 4°C, [R.C.F. = about
25 1600] HG-4L Rotor RC3 Sorval centrifuge, Dupont, Wilmington, DE).
    The primary broth was chilled at 4°C for 8 - 16 hours and
    recentrifuged at least 2 hours (conditions above) to further
    clarify the broth by removal of a putative mucopolysaccharide which
    precipitated upon standing. (An alternative processing method
30 combined both steps and involved the use of a 16 hour
    clarification centrifugation, same conditions as above.) This
    broth was then stored at 4°C prior to bioassay or filtration.

        Photorhabdus culture broth and protein toxin(s) purified from
    this broth showed activity (mortality and/or growth inhibition,
35 reduced adult emergence) against a number of insects. More
    specifically, the activity is seen against corn rootworm (larvae
    and adult), Colorado potato beetle, and turf grubs, which are
    members of the insect order *Coleoptera*. Other members of the
    Coleoptera include wireworms, pollen beetles, flea beetles, seed
40 beetles and weevils. Activity has also been observed against aster
    leafhopper, which is a member of the order, *Homoptera*. Other
    members of the *Homoptera* include planthoppers, pear pyslla, apple

sucker, scale insects, whiteflies, and spittle bugs, as well as
numerous host specific aphid species. The broth and purified
fractions are also active against beet armyworm, cabbage looper,
black cutworm, tobacco budworm, European corn borer, corn earworm,
5 and codling moth, which are members of the order *Lepidoptera*.
Other typical members of this order are clothes moth, Indian
mealmoth, leaf rollers, cabbage worm, cotton bollworm, bagworm,
Eastern tent caterpillar, sod webworm, and fall armyworm. Activity
is also seen against fruitfly and mosquito larvae, which are
10 members of the order *Diptera*. Other members of the order *Diptera*
are pea midge, carrot fly, cabbage root fly, turnip root fly, onion
fly, crane fly, house fly, and various mosquito species. Activity
is seen against carpenter ant and Argentine ant, which are members
of the order that also includes fire ants, oderous house ants, and
15 little black ants.

The broth/fraction is useful for reducing populations of
insects and were used in a method of inhibiting an insect
population. The method may comprise applying to a locus of the
insect an effective insect inactivating amount of the active
20 described. Results are reported in Table 4.

Activity against corn rootworm larvae was tested as follows.
Photorhabdus culture broth (filter sterilized, cell-free) or
purified HPLC fractions were applied directly to the surface (about
1.5 cm^2) of 0.25 ml of artificial diet in 30 μl aliquots following
25 dilution in control medium or 10 mM sodium phosphate buffer, pH
7.0, respectively. The diet plates were allowed to air-dry in a
sterile flow-hood and the wells were infested with single, neonate
Diabrotica undecimpunctata howardi (Southern corn rootworm, SCR)
hatched from sterilized eggs, with second instar SCR grown on
30 artificial diet or with second instar *Diabrotica virgifera*
virgifera (Western corn rootworm, WCR) reared on corn seedlings
grown in Metromix®. Second instar larvae were weighed prior to
addition to the diet. The plates were sealed, placed in a
humidified growth chamber and maintained at 27°C for the
35 appropriate period (4 days for neonate and adult SCR, 2-5 days for
WCR larvae, 7-14 days for second instar SCR). Mortality and weight
determinations were scored as indicated. Generally, 16 insects per
treatment were used in all studies. Control mortalities were as
follows: neonate larvae, <5%, adult beetles, 5%.
40 Activity against Colorado potato beetle was tested as follows.
Photorhabdus culture broth or control medium was applied to the
surface (about 2.0 cm^2) of 1.5 ml of standard artificial diet held
in the wells of a 24-well tissue culture plate. Each well received

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50 μl of treatment and was allowed to air dry. Individual second
instar Colorado potato beetle (*Leptinotarsa decemlineata*, CPB)
larvae were then placed onto the diet and mortality was scored
after 4 days. Ten larvae per treatment were used in all studies.
5   Control mortality was 3.3%.

Activity against Japanese beetle grubs and beetles was tested
as follows. Turf grubs (*Popillia japonica*, 2-3rd instar) were
collected from infested lawns and maintained in the laboratory in
soil/peat mixture with carrot slices added as additional diet.
10   Turf beetles were pheromone-trapped locally and maintained in the
laboratory in plastic containers with maple leaves as food.
Following application of undiluted *Photorhabdus* culture broth or
control medium to corn rootworm artificial diet (30 μl/1.54 cm²,
beetles) or carrot slices (larvae), both stages were placed singly
15   in a diet well and observed for any mortality and feeding. In both
cases there was a clear reduction in the amount of feeding (and
feces production) observed.

Activity against mosquito larvae was tested as follows. The
assay was conducted in a 96-well microtiter plate. Each well
20   contained 200 μl of aqueous solution (*Photorhabdus* culture broth,
control medium or H₂0) and approximately 20, 1-day old larvae (*Aedes
aegypti*). There were 6 wells per treatment. The results were read
at 2 hours after infestation and did not change over the three day
observation period. No control mortality was seen.
25   Activity against fruitflies was tested as follows. Purchased
Drosophila melanogaster medium was prepared using 50% dry medium
and a 50% liquid of either water, control medium or *Photorhabdus*
culture broth. This was accomplished by placing 8.0 ml of dry
medium in each of 3 rearing vials per treatment and adding 8.0 ml
30   of the appropriate liquid. Ten late instar *Drosophila melanogaster*
maggots were then added to each vial. The vials were held on a
laboratory bench, at room temperature, under fluorescent ceiling
lights. Pupal or adult counts were made after 3, 7 and 10 days of
exposure. Incorporation of *Photorhabdus* culture broth into the
35   diet media for fruitfly maggots caused a slight (17%) but
significant reduction in day-10 adult emergence as compared to
water and control medium (3% reduction).

Activity against aster leafhopper was tested as follows. The
ingestion assay for aster leafhopper (*Macrosteles severini*) is
40   designed to allow ingestion of the active without other external
contact. The reservoir for the active/"food" solution is made by
making 2 holes in the center of the bottom portion of a 35 x 10 mm
Petri dish. A 2 inch Parafilm M* square is placed across the top of

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the dish and secured with an "O" ring. A 1 oz. plastic cup is then
infested with approximately 7 leafhoppers and the reservoir is
placed on top of the cup, Parafilm down. The test solution is then
added to the reservoir through the holes. In tests using undiluted
5   Photorhabdus culture broth, the broth and control medium were
dialyzed against water to reduce control mortality. Mortality is
reported at day 2 where 26.5% control mortality was seen. In the
tests using purified fractions (200 mg protein/ml) a final
concentration of 5% sucrose was used in all treatments to improve
10   survivability of the aster leafhoppers. The assay was held in an
incubator at 28°C, 70% RH with a 16/8 photoperiod. The assay was
graded for mortality at 72 hours. Control mortality was 5.5%.

Activity against Argentine ants was tested as follows. A 1.5
ml aliquot of 100% Photorhabdus culture broth, control medium or
15   water was pipetted into 2.0 ml clear glass vials. The vials were
plugged with a piece of cotton dental wick that was moistened with
the appropriate treatment. Each vial was placed into a separate
60x16mm Petri dish with 8 to 12 adult Argentine ants (Linepithema
humile). There were three replicates per treatment. Bioassay
20   plates were held on a laboratory bench, at room temperature under
fluorescent ceiling lights. Mortality readings were made after 5
days of exposure. Control mortality was 24%.

Activity against carpenter ant was tested as follows. Black
carpenter ant workers (Camponotus pennsylvanicus) were collected
25   from trees on DowElanco property in Indianapolis, IN. Tests with
Photorhabdus culture broth were performed as follows. Each plastic
bioassay container (7 1/8" x 3") held fifteen workers, a paper
harborage and 10 ml of broth or control media in a plastic shot
glass. A cotton wick delivered the treatment to the ants through a
30   hole in the shot glass lid. All treatments contained 5% sucrose.
Bioassays were held in the dark at room temperature and graded at
19 days. Control mortality was 9%. Assays delivering purified
fractions utilized artificial ant diet mixed with the treatment
(purified fraction or control solution) at a rate of 0.2 ml
35   treatment/2.0 g diet in a plastic test tube. The final protein
concentration of the purified fraction was less than 10 µg/g diet.
Ten ants per treatment, a water source, harborage and the treated
diet were placed in sealed plastic containers and maintained in the
dark at 27°C in a humidified incubator. Mortality was scored at
40   day 10. No control mortality was seen.

Activity against various lepidopteran larvae was tested as
follows. Photorhabdus culture broth or purified fractions were

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applied directly to the surface (about 1.5 cm^2) of 0.25 ml of
standard artificial diet in 30 μl aliquots following dilution in
control medium or 10 mM sodium phosphate buffer, pH 7.0,
respectively. The diet plates were allowed to air-dry in a sterile
5   flow-hood and the wells were infested with single, neonate larva.
European corn borer (*Ostrinia nubilalis*) and corn earworm
(*Helicoverpa zea*) eggs were supplied from commercial sources and
hatched in-house, whereas beet armyworm (*Spodoptera exigua*),
cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis
10 virescens*), codling moth (*Laspeyresia pomonella*) and black cutworm
(*Agrotis ipsilon*) larvae were supplied internally. Following
infestation with larvae, the diet plates were sealed, placed in a
humidified growth chamber and maintained in the dark at 27°C for
the appropriate period. Mortality and weight determinations were
15 scored at days 5-7 for *Photorhabdus* culture broth and days 4-7 for
the purified fraction. Generally, 16 insects per treatment were
used in all studies. Control mortality ranged from 4-12.5% for
control medium and was less than 10% for phosphate buffer.

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Table 4

Effect of *Photorhabdus luminescens* (Strain W-14)

Culture Broth and Purified Toxin Fraction on Mortality and Growth

Inhibition of Different Insect Orders/Species

5

Insect Order/Species	Broth		Purified Fraction	
	% Mort.	% G.I.	% Mort.	% G.I.
COLEOPTERA				
Corn Rootworm				
Southern/neonate larva	100	na	100	na
Southern/2nd instar	na	38.5	nt	nt
Southern/adult	45	nt	nt	nt
Western/2nd instar	na	35	nt	nt
Colorado Potato Beetle	93	nt	nt	nt
2nd instar				
Turf Grub	na	a.f.	nt	nt
3rd instar	na	a.f.	nt	nt
adult				
DIPTERA				
Fruit Fly (adult emergence)	17	nt	nt	nt
Mosquito larvae	100	na	nt	nt
HOMOPTERA				
Aster Leafhopper	96.5	na	100	na
HYMENOPTERA				
Argentine Ant	75	na	nt	na
Carpenter Ant	71	na	100	na
LEPIDOPTERA				
Beet Armyworm	12.5	36	18.75	41.4
Black Cutworm	nt	nt	0	71.2
Cabbage Looper	nt	nt	21.9	66.8
Codling Moth	nt	nt	6.25	45.9
Corn Earworm	56.3	94.2	97.9	na
European Corn Borer	96.7	98.4	100	na
Tobacco Budworm	13.5	52.5	19.4	85.6

Mort. = mortality, G.I. = growth inhibition,
na = not applicable, nt = not tested, a.f. = anti-feedant

Example 3

Insecticide Utility upon Soil Application

10

Photorhabdus luminescens (strain W-14) culture broth was shown
to be active against corn rootworm when applied directly to soil or
a soil-mix (Metromix®). Activity against neonate SCR and WCR in

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Metromix® was tested as follows (Table 5). The test was run using
corn seedlings (United Agriseeds brand CL614) that were germinated
in the light on moist filter paper for 6 days. After roots were
approximately 3-6 cm long, a single kernel/seedling was planted in

5   a 591 ml clear plastic cup with 50 gm of dry Metromix®. Twenty
neonate SCR or WCR were then placed directly on the roots of the
seedling and covered with Metromix®. Upon infestation, the
seedlings were then drenched with 50 ml total volume of a diluted
broth solution. After drenching, the cups were sealed and left at

10   room temperature in the light for 7 days. Afterwards, the
seedlings were washed to remove all Metromix® and the roots were
excised and weighed. Activity was rated as the percentage of corn
root remaining relative to the control plants and as leaf damage
induced by feeding. Leaf damage was scored visually and rated as

15   either -, +, ++, or +++, with - representing no damage and +++
representing severe damage.

        Activity against neonate SCR in soil was tested as follows
(Table 6). The test was run using corn seedlings (United Agriseeds
brand CL614) that were germinated in the light on moist filter

20   paper for 6 days. After the roots were approximately 3-6 cm long,
a single kernel/seedling was planted in a 591 ml clear plastic cup
with 150 gm of soil from a field in Lebanon, IN planted the
previous year with corn. This soil had not been previously treated
with insecticides. Twenty neonate SCR were then placed directly on

25   the roots of the seedling and covered with soil. After
infestation, the seedlings were drenched with 50 ml total volume of
a diluted broth solution. After drenching, the unsealed cups were
incubated in a high relative humidity chamber (80%) at 78°F.
Afterwards, the seedlings were washed to remove all soil and the

30   roots were excised and weighed. Activity was rated as the
percentage of corn root remaining relative to the control plants
and as leaf damage induced by feeding. Leaf damage was scored
visually and rated as either -, +, ++, or +++, with - representing
no damage and +++ representing severe damage.

35

<u>Table 5</u>
<u>Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on
Rootworm Larvae after Post-Infestation Drenching (Metromix®)</u>

	Treatment	Larvae	Leaf Damage	Root Weight (g)	%
5	**Southern Corn Rootworm**				
	Water	—	—	0.4916 ± 0.023	100
	Medium (2.0% v/v)	—	—	0.4416 ± 0.029	100
10	Broth (6.25%v/v)	—	—	0.4641 ± 0.081	100
	Water	+	+++	0.1410 ± 0.006	28.7
	Media (2.0% v/v)	+	+++	0.1345 ± 0.028	30.4
15	Broth (1.56% v/v)	+	—	0.4830 ± 0.031	104
	Western Corn Rootworm				
	Water	—	—	0.4446 ± 0.019	100
	Broth (2.0% v/v)	—	—	0.4069 ± 0.026	100
20	Water	+	—	0.2202 ± 0.015	49
	Broth (2.0% v/v)	+	—	0.3879 ± 0.013	95

25

<u>Table 6</u>
<u>Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on
Southern Corn Rootworm Larvae after Post-Infestation Drenching
(Soil)</u>

	Treatment	Larvae	Leaf Damage	Root Weight(g)	%
30	Water	—	—	0.2148 ± 0.014	100
	Broth (50% v/v)	—	—	0.2260 ± 0.016	103
35	Water	+	+++	0.0916 ± 0.009	43
	Broth (50% v/v)	+	—	0.2428 ± 0.032	113

Activity of *Photorhabdus luminescens* (strain W-14) culture
broth against second instar turf grubs in Metromix® was observed in
40   tests conducted as follows (Table 7). Approximately 50 gm of dry
Metromix® was added to a 591 ml clear plastic cup. The Metromix®
was then drenched with 50 ml total volume of a 50% (v/v) diluted
Photorhabdus broth solution. The dilution of crude broth was made
with water, with 50% broth being prepared by adding 25 ml of crude
45   broth to 25 ml of water for 50 ml total volume. A 1% (w/v)
solution of proteose peptone #3 (PP3), which is a 50% dilution of
the normal media concentration, was used as a broth control. After
drenching, five second instar turf grubs were placed on the top of
the moistened Metromix®. Healthy turf grub larvae burrowed rapidly
50   into the Metromix®. Those larvae that did not burrow within 1h were

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removed and replaced with fresh larvae. The cups were sealed and
placed in a 28°C incubator, in the dark. After seven days, larvae
were removed from the Metromix° and scored for mortality. Activity
was rated the percentage of mortality relative to control.

Table 7
Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on
Turf Grub after Pre-Infestation Drenching (Metromix°)

Treatment	Mortality*	Mortality %
Water	7/15	47
Control medium (1.0% w/v)	12/19	63
Broth (50% v/v)	17/20	85

*expressed as a ratio of dead/living larvae

Example 4
Insecticide Utility upon Leaf Application

Activity of *Photorhabdus* broth against European corn borer was
seen when the broth was applied directly to the surface of maize
leaves (Table 8). In these assays *Photorhabdus* broth was diluted
100-fold with culture medium and applied manually to the surface of
excised maize leaves at a rate of about 6.0 μl/cm^2 of leaf surface.
The leaves were air dried and cut into equal sized strips
approximately 2 x 2 inches. The leaves were rolled, secured with
paper clips and placed in 1 oz plastic shot glasses with 0.25 inch
of 2% agar on the bottom surface to provide moisture. Twelve
neonate European corn borers were then placed onto the rolled leaf
and the cup was sealed. After incubation for 5 days at 27°C in the
dark, the samples were scored for feeding damage and recovered
larvae.

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Table 8

Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on
European Corn Borer Larvae Following Pre-Infestation Application to
Excised Maize Leaves

Treatment	Leaf Damage	Larvae Recovered	Weight (mg)
Water	Extensive	55/120	0.42 mg
Control Medium	Extensive	40/120	0.50 mg
Broth (1.0% v/v)	Trace	3/120	0.15 mg

Activity of the culture broth against neonate tobacco budworm
(*Heliothis virescens*) was demonstrated using a leaf dip
methodology. Fresh cotton leaves were excised from the plant and
leaf disks were cut with an 18.5 mm cork-borer. The disks were
individually emersed in control medium (PP3) or *Photorhabdus
luminescens* (strain W-14) culture broth which had been concentrated
approximately 10-fold using an Amicon (Beverly, MA), Proflux M12
tangential filtration system with a 10 kDa filter. Excess liquid
was removed and a straightened paper clip was placed through the
center of the disk. The paper clip was then wedged into a plastic,
1.0 oz shot glass containing approximately 2.0 ml of 1% Agar. This
served to suspend the leaf disk above the agar. Following drying
of the leaf disk, a single neonate tobacco budworm larva was placed
on the disk and the cup was capped. The cups were then sealed in a
plastic bag and placed in a darkened, 27°C incubator for 5 days.
At this time the remaining larvae and leaf material were weighed to
establish a measure of leaf damage (Table 9).

Table 9

Effect of *Photorhabdus luminescens* (Strain W-14) Culture Broth on
Tobacco Budworm Neonates in a Cotton-Leaf Dip Assay

		Final Weights (mg)
Treatment	Leaf Disk	Larvae
Control leaves	55.7 ± 1.3	na*
Control Medium	34.0 ± 2.9	4.3 ± 0.91
Photorhabdus broth	54.3 ± 1.4	0.0**

* - not applicable, ** - no live larvae found

Example 5, Part A

Characterization of Toxin Peptide Components

In a subsequent analysis, the toxin protein subunits of the
bands isolated as in Example 1 were resolved on a 7% SDS

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polyacrylamide electrophoresis gel with a ratio of 30:0.8
(acrylamide:BIS-acrylamide). This gel matrix facilitates better
resolution of the larger proteins. The gel system used to estimate
the Band 1 and Band 2 subunit molecular weights in Example 1 was an
5   18% gel with a ratio of 38:0.18 (acrylamide:BIS-acrylamide), which
allowed for a broader range of size separation, but less resolution
of higher molecular weight components.

In this analysis, 10, rather than 8, protein bands were
resolved. Table 10 reports the calculated molecular weights of the
10   10 resolved bands, and directly compares the molecular weights
estimated under these conditions to those of the prior example. It
is not surprising that additional bands were detected under the
different separation conditions used in this example. Variations
between the prior and new estimates of molecular weight are also to
15   be expected given the differences in analytical conditions. In the
analysis of this example, it is thought that the higher molecular
weight estimates are more accurate than in Example 1, as a result
of improved resolution. However, these are estimates based on SDS
PAGE analysis, which are typically not analytically precise and
20   result in estimates of peptides and which may have been further
altered due to post- and co-translational modifications.

Amino acid sequences were determined for the N-terminal
portions of five of the 10 resolved peptides. Table 10
+ correlates the molecular weight of the proteins and the
25   identified sequences. In SEQ ID NO:2, certain analyses suggest
that the proline at residue 5 may be an asparagine (asn). In SEQ
ID NO:3, certain analyses suggest that the amino acid residues at
positions 13 and 14 are both arginine (arg). In SEQ ID NO:4,
certain analyses suggest that the amino acid residue at position 6
30   may be either alanine (ala) or serine (ser). In SEQ ID NO:5,
certain analyses suggest that the amino acid residue at position 3
may be aspartic acid (asp).

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<u>Table 10</u>

ESTIMATE	NEW ESTIMATE*	SEQ. LISTING
208	200.2 kDa	SEQ ID NO:1
184	175.0 kDa	SEQ ID NO:2
65.6	68.1 kDa	SEQ ID NO:3
60.8	65.1 kDa	SEQ ID NO:4
56.2	58.3 kDa	SEQ ID NO:5
25.1	23.2 kDa	SEQ ID NO:15

*New estimates are based on SDS PAGE and are not based on
gene sequences. SDS PAGE is not analytically precise.

<u>Example 5, Part B</u>
<u>Characterization of Toxin Peptide Components</u>

New N-terminal sequence, SEQ ID NO:15, Ala Gln Asp Gly Asn Gln
Asp Thr Phe Phe Ser Gly Asn Thr, was obtained by further N-terminal
sequencing of peptides isolated from Native HPLC-purified toxin as
described in Example 5, Part A, above. This peptide comes from the
$tcaA$ gene. The peptide labeled TcaA$_{ii}$, starts at position 254 and
goes to position 491, where the TcaA$_{iii}$ peptide starts, SEQ ID
NO:4. The estimated size of the peptide based on the gene sequence
is 25,240 Da.

<u>Example 6</u>
<u>Characterization of Toxin Peptide Components</u>

In yet another analysis, the toxin protein complex was re-
isolated from the *Photorhabdus luminescens* growth medium (after
culture without Tween) by performing a 10% - 80% ammonium sulfate
precipitation followed by an ion exchange chromatography step (Mono
Q) and two molecular sizing chromatography steps. These conditions
were like those used in Example 1. During the first molecular
sizing step, a second biologically active peak was found at about
100 ± 10 kDa. Based upon protein measurements, this fraction was
20 - 50 fold less active than the larger, or primary, active peak
of about 860 ± 100 kDa (native). During this isolation experiment,
a smaller active peak of about 325 ± 50 kDa that retained a
considerable portion of the starting biological activity was also
resolved. It is thought that the 325 kDa peak is related to or
derived from the 860 kDa peak.
A 56 kDa protein was resolved in this analysis. The N-
terminal sequence of this protein is presented in SEQ ID NO:6. It

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is noteworthy that this protein shares significant identity and
conservation with SEQ ID NO:5 at the N-terminus, suggesting that
the two may be encoded by separate members of a gene family and
that the proteins produced by each gene are sufficiently similar to
5   both be operable in the insecticidal toxin complex.

A second, prominent 185 kDa protein was consistently present
in amounts comparable to that of protein 3 from Table 10, and may
be the same protein or protein fragment. The N-terminal sequence
of this 185 kDa protein is shown at SEQ ID NO:7.

10   Additional N-terminal amino acid sequence data were also
obtained from isolated proteins. None of the determined N-terminal
sequences appear identical to a protein identified in Table 10.
Other proteins were present in isolated preparation. One such
protein has an estimated molecular weight of 108 kDa and an N-
15   terminal sequence as shown in SEQ ID NO:8. A second such protein
has an estimated molecular weight of 80 kDa and an N-terminal
sequence as shown in SEQ ID NO:9.

When the protein material in the approximately 325 kDa active
peak was analyzed by size, bands of approximately 51, 31, 28, and
20   22 kDa were observed. As in all cases in which a molecular weight
was determined by analysis of electrophoretic mobility, these
molecular weights were subject to error effects introduced by
buffer ionic strength differences, electrophoresis power
differences, and the like. One of ordinary skill would understand
25   that definitive molecular weight values cannot be determined using
these standard methods and that each was subject to variation. It
was hypothesized that proteins of these sizes are degradation
products of the larger protein species (of approximately 200 kDa
size) that were observed in the larger primary toxin complex.

30   Finally, several preparations included a protein having the N-
terminal sequence shown in SEQ ID NO:10. This sequence was
strongly homologous to known chaperonin proteins, accessory
proteins known to function in the assembly of large protein
complexes. Although the applicants could not ascribe such an
35   assembly function to the protein identified in SEQ ID NO:10, it was
consistent with the existence of the described toxin protein
complex that such a chaperonin protein could be involved in its
assembly. Moreover, although such proteins have not directly been
suggested to have toxic activity, this protein may be important to
40   determining the overall structural nature of the protein toxin, and
thus, may contribute to the toxic activity or durability of the
complex in vivo after oral delivery.

Subsequent analysis of the stability of the protein toxin
complex to proteinase K was undertaken. It was determined that
after 24 hour incubation of the complex in the presence of a 10-
fold molar excess of proteinase K, activity was virtually

5   eliminated (mortality on oral application dropped to about 5%).
These data confirm the proteinaceous nature of the toxin.

The toxic activity was also retained by a dialysis membrane,
again confirming the large size of the native toxin complex.

10   Example 7
             Isolation, Characterization and Partial Amino Acid
                       Sequencing of *Photorhabdus* Toxins

Isolation and N-Terminal Amino Acid Sequencing

15   In a set of experiments conducted in parallel to Examples 5
and 6, ammonium sulfate precipitation of *Photorhabdus* proteins was
performed by adjusting *Photorhabdus* broth, typically 2-3 liters, to
a final concentration of either 10% or 20% by the slow addition of
ammonium sulfate crystals. After stirring for 1 hour at 4°C, the

20   material was centrifuged at 12,000 x g for 30 minutes. The
supernatant was adjusted to 80% ammonium sulfate, stirred at 4°C
for 1 hour, and centrifuged at 12,000 x g for 60 minutes. The
pellet was resuspended in one-tenth the volume of 10 mM $Na_2 PO_4$, pH
7.0 and dialyzed against the same phosphate buffer overnight at

25   4°C. The dialyzed material was centrifuged at 12,000 x g for 1
hour prior to ion exchange chromatography.

A HR 16/50 Q Sepharose (Pharmacia) anion exchange column was
equilibrated with 10 mM $Na_2 PO_4$, pH 7.0. Centrifuged, dialyzed
ammonium sulfate pellet was applied to the Q Sepharose column at a

30   rate of 1.5 ml/min and washed extensively at 3.0 ml/min with
equilibration buffer until the optical density (O.D. 280) reached
less than 0.100. Next, either a 60 minute NaCl gradient ranging
from 0 to 0.5 M at 3 ml/min, or a series of step elutions using 0.1
M, 0.4 M and finally 1.0 NaCl for 60 minutes each was applied to

35   the column. Fractions were pooled and concentrated using a
Centriprep 100. Alternatively, proteins could be eluted by a
single 0.4 M NaCl wash without prior elution with 0.1 M NaCl.

Two milliliter aliquots of concentrated Q Sepharose samples
were loaded at 0.5 ml/min onto a HR 16/50 Superose 12 (Pharmacia)

40   gel filtration column equilibrated with 10 mM $Na_2 PO_4$, pH 7.0. The
column was washed with the same buffer for 240 min at 0.5 ml/min
and 2 min samples were collected. The void volume material was

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collected and concentrated using a Centriprep 100. Two milliliter
aliquots of concentrated Superose 12 samples were loaded at 0.5
ml/min onto a HR 16/50 Sepharose 4B-CL (Pharmacia) gel filtration
column equilibrated with 10 mM $Na_2\ PO_4$, pH 7.0. The column was

5   washed with the same buffer for 240 min at 0.5 ml/min and 2 min
samples were collected.

The excluded protein peak was subjected to a second
fractionation by application to a gel filtration column that used a
Sepharose CL-4B resin, which separates proteins ranging from about

10   30 kDa to 1000 kDa. This fraction was resolved into two peaks; a
minor peak at the void volume (>1000 kDa) and a major peak which
eluted at an apparent molecular weight of about 860 kDa. Over a
one week period subsequent samples subjected to gel filtration
showed the gradual appearance of a third peak (approximately 325

15   kDa) that seemed to arise from the major peak, perhaps by limited
proteolysis. Bioassays performed on the three peaks showed that
the void peak had no activity, while the 860 kDa toxin complex
fraction was highly active, and the 325 kDa peak was less active,
although quite potent. SDS PAGE analysis of Sepharose CL-4B toxin

20   complex peaks from different fermentation productions revealed two
distinct peptide patterns, denoted "P" and "S". The two patterns
had marked differences in the molecular weights and concentrations
of peptide components in their fractions. The "S" pattern,
produced most frequently, had 4 high molecular weight peptides

25   (> 150 kDa) while the "P" pattern had 3 high molecular weight
peptides. In addition, the "S" peptide fraction was found to have
2-3 fold more activity against European Corn Borer. This shift may
be related to variations in protein expression due to age of
inoculum and/or other factors based on growth parameters of aged

30   cultures.

Milligram quantities of peak toxin complex fractions
determined to be "P" or "S" peptide patterns were subjected to
preparative SDS PAGE, and transblotted with TRIS-glycine
(Seprabuff™ to PVDF membranes (ProBlott™, Applied Biosystems) for

35   3-4 hours. Blots were sent for amino acid analysis and N-terminal
amino acid sequencing at Harvard MicroChem and Cambridge ProChem,
respectively. Three peptides in the "S" pattern had unique N-
terminal amino acid sequences compared to the sequences identified
in the previous example. A 201 kDa (TcdA$_{ii}$) peptide set forth as

40   SEQ ID NO:13 below shared between 33% amino acid identity and 50%
similarity (similarity and identity were calculated by hand) with
SEQ ID NO:1 (TcbA$_{ii}$)(in Table 10 vertical lines denote amino acid

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identities and colons indicate conservative amino acid
substitutions). A second peptide of 197 kDa, SEQ ID NO:14 (TcdB),
had 42% identity and 58% similarity with SEQ ID NO:2 (TcaC)
(similarity and identity were calculated by hand). Yet a third
5   peptide of 205 kDa was denoted TcdA$_{ii}$. In addition, a limited N-
terminal amino acid sequence, SEQ ID NO:16 (TcbA), of a peptide of
at least 235 kDa was identical with the amino acid sequence, SEQ ID
NO:12, deduced from a cloned gene (tcbA), SEQ ID NO:11, containing
a deduced amino acid sequence corresponding to SEQ ID NO:1
10   (TcbA$_{ii}$). This indicates that the larger 235+ kDa peptide was
proteolytically processed to the 201 kDa peptide, (TcbA$_{ii}$), (SEQ ID
NO:1) during fermentation, possibly resulting in activation of the
molecule. In yet another sequence, the sequence originally
reported as SEQ ID NO:5 (TcaB$_{ii}$) reported in Example 5 above, was
15   found to contain an aspartic acid residue (Asp) at the third
position rather than glycine (Gly) and two additional amino acids
Gly and Asp at the eighth and ninth positions, respectively. In
yet two other sequences, SEQ ID NO:2 (TcaC) and SEQ ID NO:3
(TcaB$_{i}$), additional amino acid sequence was obtained.

20   Densitometric quantitation was performed using a sample that was
identical to the "S" preparation sent for N-terminal analysis.
This analysis showed that the 201 kDa and 197 kDa peptides
represent 7.0% and 7.2%, respectively, of the total Coomassie
brilliant blue stained protein in the "S" pattern and are present in
25   amounts similar to the other abundant peptides. It was speculated
that these peptides may represent protein homologs, analogous to
the situation found with other bacterial toxins, such as various
CryI Bt toxins. These proteins vary from 40-90% similarity at their
N-terminal amino acid sequence, which encompasses the toxic
30   fragment.

Internal Amino Acid Sequencing
        To facilitate cloning of toxin peptide genes, internal amino
acid sequences of selected peptides were obtained as followed.
35   Milligram quantities of peak 2A fractions determined to be "P" or
"S" peptide patterns were subjected to preparative SDS PAGE, and
transblotted with TRIS-glycine (Seprabuff™ to PVDF membranes
(ProBlott™, Applied Biosystems) for 3-4 hours. Blots were sent for
amino acid analysis and N-terminal amino acid sequencing at Harvard
40   MicroChem and Cambridge ProChem, respectively. Three peptides,
referred to as TcbA$_{ii}$ (containing SEQ ID NO:1), TcdA$_{ii}$, and TcaB$_{i}$
(containing SEQ ID NO:3) were subjected to trypsin digestion by

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Harvard MicroChem followed by HPLC chromatography to separate
individual peptides. N-terminal amino acid analysis was performed
on selected tryptic peptide fragments. Two internal peptides were
sequenced for the peptide TcdA$_{ii}$ (205 kDa peptide) referred to as

5   TcdA$_{ii}$-PT111 (SEQ ID NO:17) and TcdA$_{ii}$-PT79 (SEQ ID NO:18). Two
internal peptides were sequenced for the peptide TcaB$_i$ (68 kDa
peptide) referred to as TcaB$_i$-PT158 (SEQ ID NO:19) and TcaB$_i$-PT108
(SEQ ID NO:20). Four internal peptides were sequenced for the
peptide TcbA$_{ii}$ (201 kDa peptide) referred to as TcbA$_{ii}$-PT103 (SEQ

10 ID NO:21), TcbA$_{ii}$-PT56 (SEQ ID NO:22), TcbA$_{ii}$-PT81(a) (SEQ ID
NO:23), and TcbA$_{ii}$-PT81(b) (SEQ ID NO:24).

<u>Table 11</u>
<u>N-Terminal Amino Acid Sequences</u>

15   (similarity and identity were calculated by hand)

201 kDa (33% identity & 50% similarity to SEQ ID NO.1)
    L I G Y N N Q F S G * A   SEQ ID NO:13
       : | | | : |
20 F I Q G Y S D L F G - A   SEQ ID NO:1

197 kDa (42% identity & 58% similarity SEQ ID NO.2)
M Q N S Q T F S V G E L SEQ ID NO.14
| | : | | : : |
25 M Q D S P E V S I T T L SEQ ID NO.2

<u>Example 8</u>
<u>Construction of a Cosmid Library of Photorhabdus luminescens W-14</u>
30 <u>Genomic DNA and its Screening to Isolate Genes Encoding Peptides</u>
<u>Comprising the Toxic Protein Preparation</u>

As a prerequisite for the production of Photorhabdus insect
toxic proteins in heterologous hosts, and for other uses, it is
35 necessary to isolate and characterize the genes that encode those
peptides. This objective was pursued in parallel. One approach,
described later, was based on the use of monoclonal and polyclonal
antibodies raised against the purified toxin which were then used
to isolate clones from an expression library. The other approach,
40 described in this example, is based on the use of the N-terminal
and internal amino acid sequence data to design degenerate
oligonucleotides for use in PCR amplification. Either method can be
used to identify DNA clones that contain the peptide-encoding genes
so as to permit the isolation of the respective genes, and the
45 determination of their DNA base sequence.

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Genomic DNA Isolation

       Photorhabdus luminescens strain W-14 (ATCC accession number
55397) was grown on 2% proteose peptone #3 agar (Difco
Laboratories, Detroit, MI) and insecticidal toxin competence was

5   maintained by repeated bioassay after passage, using the method
described in Example 1 above. A 50 ml shake culture was produced
in a 175 ml baffled flask in 2% proteose peptone #3 medium, grown
at 28°C and 150 rpm for approximately 24 hours. 15 ml of this
culture was pelleted and frozen in its medium at -20°C until it was

10   thawed for DNA isolation. The thawed culture was centrifuged, (700
x g, 30 min) and the floating orange mucopolysaccharide material
was removed. The remaining cell material was centrifuged (25,000 x
g, 15 min) to pellet the bacterial cells, and the medium was
removed and discarded.

15   Genomic DNA was isolated by an adaptation of the CTAB method
described in section 2.4.1 of Current Protocols in Molecular
Biology (Ausubel *et al.* eds, John Wiley & Sons, 1994) [modified to
include a salt shock and with all volumes increased 10-fold]. The
pelleted bacterial cells were resuspended in TE buffer (10 mM Tris-

20   HCl, 1 mM EDTA, pH 8.0) to a final volume of 10 ml, then 12 ml of 5
M NaCl was added; this mixture was centrifuged 20 min at 15,000 x
g. The pellet was resuspended in 5.7 ml TE and 300 ml of 10% SDS
and 60 ml of 20 mg/ml proteinase K (Gibco BRL Products, Grand
Island, NY; in sterile distilled water) were added to the

25   suspension. This mixture was incubated at 37°C for 1 hr; then
approximately 10 mg lysozyme (Worthington Biochemical Corp.,
Freehold, NJ) was added. After an additional 45 min, 1 ml of 5 M
NaCl and 800 ml of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl)
were added. This preparation was incubated 10 min at 65°C, then

30   gently agitated and further incubated and agitated for
approximately 20 min to assist clearing of the cellular material.
An equal volume of chloroform/isoamyl alcohol solution (24:1, v/v)
was added, mixed gently and centrifuged. After two extractions
with an equal volume of PCI (phenol/chloroform/isoamyl alcohol;

35   50:49:1, v/v/v; equilibrated with 1 M Tris-HCl, pH 8.0;
Intermountain Scientific Corporation, Kaysville, UT), the DNA was
precipitated with 0.6 volume of isopropanol. The DNA precipitate
was gently removed with a glass rod, washed twice with 70% ethanol,
dried, and dissolved in 2 ml STE (10 mM Tris-HCl pH 8.0, 10 mM

40   NaCl, 1 mM EDTA). This preparation contained 2.5 mg/ml DNA, as
determined by optical density at 260 nm (i.e., OD_{260}).

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The molecular size range of the isolated genomic DNA was
evaluated for suitability for library construction. CHEF gel
analysis was performed in 1.5% agarose (Seakem° LE, FMC BioProducts,
Rockland, ME) gels with 0.5 X TBE buffer (44.5 mM Tris-HCl pH 8.0,
5   44.5 mM H_3BO_3, 1 mM EDTA) on a BioRad CHEF-DR II apparatus with a
Pulsewave 760 Switcher (Bio-Rad Laboratories, Inc., Richmond, CA).
The running parameters were: initial A time, 3 sec; final A time,
12 sec; 200 volts; running temperature, 4-18°C; run time, 16.5 hr.
Ethidium bromide staining and examination of the gel under
10   ultraviolet light indicated the DNA ranged from 30-250 kbp in size.

Construction of Library

A partial Sau3A 1 digest was made of this Photorhabdus genomic
DNA preparation. The method was based on section 3.1.3 of Ausubel
15   (supra.). Adaptions included running smaller scale reactions under
various conditions until nearly optimal results were achieved.
Several scaled-up large reactions with varied conditions were run,
the results analyzed on CHEF gels, and only the best large scale
preparation was carried forward. In the optimal case, 200 μg of
20   Photorhabdus genomic DNA was incubated with 1.5 units of Sau3A 1
(New England Biolabs, "NEB", Beverly, MA) for 15 min at 37°C in 2
ml total volume of 1X NEB 4 buffer (supplied as 10X by the
manufacturer). The reaction was stopped by adding 2 ml of PCI and
centrifuging at 8000 x g for 10 min. To the supernatant were added
25   200 μl of 5 M NaCl plus 6 ml of ice-cold ethanol. This preparation
was chilled for 30 min at -20°C, then centrifuged at 12,000 x g for
15 min. The supernatant was removed and the precipitate was dried
in a vacuum oven at 40°C, then resuspended in 400 μl STE.
Spectrophotometric assay indicated about 40% recovery of the input
30   DNA. The digested DNA was size fractionated on a sucrose gradient
according to section 5.3.2 of CPMB (op. cit.). A 10% to 40% (w/v)
linear sucrose gradient was prepared with a gradient maker in
Ultra-Clear™ tubes (Beckman Instruments, Inc., Palo Alto, CA) and
the DNA sample was layered on top. After centrifugation, (26,000
35   rpm, 17 hr, Beckman SW41 rotor, 20°C), fractions (about 750 μl)
were drawn from the top of the gradient and analyzed by CHEF gel
electrophoresis (as described earlier). Fractions containing Sau3A
1 fragments in the size range 20-40 kbp were selected and DNA was
precipitated by a modification (amounts of all solutions increased
40   approximately 6.3-fold) of the method in section 5.3.3 of Ausubel
(supra.). After overnight precipitation, the DNA was collected by
centrifugation (17,000 x g, 15 min), dried, redissolved in TE,

pooled into a final volume of 80 µl,. and reprecipitated with the
addition of 8 µl 3 M sodium acetate and 220 µl ethanol. The pellet
collected by centrifugation as above was resuspended in 12 µl TE.
Concentration of the DNA was determined by Hoechst 33258 dye

5   (Polysciences, Inc., Warrington, PA) fluorometry in a Hoefer TKO100
fluorimeter (Hoefer Scientific Instruments, San Francisco, CA).
Approximately 2.5 µg of the size-fractionated DNA was recovered.

        Thirty µg of cosmid pWE15 DNA (Stratagene, La Jolla, CA) was
digested to completion with 100 units of restriction enzyme BamH 1

10   (NEB) in the manufacturer's buffer (final volume of 200 µl, 37°C, 1
hr). The reaction was extracted with 100 µl of PCI and DNA was
precipitated from the aqueous phase by addition of 20 µl 3M sodium
acetate and 550 µl -20°C absolute ethanol. After 20 min at -70°C,
the DNA was collected by centrifugation (17,000 x g, 15 min), dried

15   under vacuum, and dissolved in 180 µl of 10 mM Tris-HCl, pH 8.0.
To this were added 20 µl of 10X CIP buffer (100 mM Tris-HCl, pH
8.3; 10 mM ZnCl$_2$; 10 mM MgCl$_2$), and 1 µl (0.25 units) of 1:4 diluted
calf intestinal alkaline phosphatase (Boehringer Mannheim
Corporation, Indianapolis, IN). After 30 min at 37°C, the

20   following additions were made: 2 µl 0.5 M EDTA, pH 8.0; 10 µl 10%
SDS; 0.5 µl of 20 mg/ml proteinase K (as above), followed by
incubation at 55°C for 30 min. Following sequential extractions
with 100 µl of PCI and 100 µl phenol (Intermountain Scientific
Corporation, equilibrated with 1 M Tris-HCl, pH 8.0), the

25   dephosphorylated DNA was precipitated by addition of 72 µl of 7.5 M
ammonium acetate and 550 µl -20°C ethanol, incubation on ice for 30
min, and centrifugation as above. The pelleted DNA was washed once
with 500 µl -20°C 70% ethanol, dried under vacuum, and dissolved in
20 µl of TE buffer.

30   Ligation of the size-fractionated Sau3A 1 fragments to the
BamH 1-digested and phosphatased pWE15 vector was accomplished
using T4 ligase (NEB) by a modification (i.e., use of premixed 10X
ligation buffer supplied by the manufacturer) of the protocol in
section 3.33 of Ausubel. Ligation was carried out overnight in a

35   total volume of 20 µl at 15°C, followed by storage at - 20°C.

        Four µl of the cosmid DNA ligation reaction, containing about
1 µg of DNA, was packaged into bacteriophage lambda using a
commercial packaging extract (Gigapack® III Gold Packaging Extract,
Stratagene), following the manufacturer's directions. The packaged

40   preparation was stored at 4°C until use. The packaged cosmid
preparation was used to infect Escherichia coli XL1 Blue MR cells

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(Stratagene) according to the Gigapack® III Gold protocols
("Titering the Cosmid Library"), as follows. XLl Blue MR cells
were grown in LB medium (g/L: Bacto-tryptone, 10; Bacto-yeast
extract, 5; Bacto-agar, 15; NaCl, 5; [Difco Laboratories, Detroit,
5   MI]) containing 0.2% (w/v) maltose plus 10 mM $MgSO_4$, at 37°C. After
5 hr growth, cells were pelleted at 700 x g (15 min) and
resuspended in 6 ml of 10 mM $MgSO_4$. The culture density was
adjusted with 10 mM $MgSO_4$ to OD_{600} = 0.5. The packaged cosmid
library was diluted 1:10 or 1:20 with sterile SM medium (0.1 M
10   NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl pH 7.5, 0.01% w/v gelatin), and 25
µl of the diluted preparation was mixed with 25 µl of the diluted
XLl Blue MR cells. The mixture was incubated at 25°C for 30 min
(without shaking), then 200 µl of LB broth was added, and
incubation was continued for approximately 1 hr with occasional
15   gentle shaking. Aliquots (20-40 µl) of this culture were spread on
LB agar plates containing 100 mg/1 ampicillin (i.e., $LB-Amp_{100}$) and
incubated overnight at 37°C. To store the library without
amplification, single colonies were picked and inoculated into
individual wells of sterile 96-well microwell plates; each well
20   containing 75 µl of Terrific Broth (TB media: 12 g/1 Bacto-
tryptone, 24 g/1 Bacto-yeast extract, 0.4% v/v glycerol, 17 mM
KH_2PO_4, 72 mM K_2HPO_4) plus 100 mg/1 ampicillin (i.e., $TB-Amp_{100}$) and
incubated (without shaking) overnight at 37°C. After replicating
the 96-well plate into a copy plate, 75 µl/well of filter-
25   sterilized TB:glycerol (1:1, v/v; with, or without, 100 mg/1
ampicillin) was added to the plate, it was shaken briefly at 100
rpm, 37°C, and then closed with Parafilm® (American National Can,
Greenwich, CT) and placed in a -70°C freezer for storage. Copy
plates were grown and processed identically to the master plates.
30   A total of 40 such master plates (and their copies) were prepared.

Screening of the Library with Radiolabeled DNA Probes
        To prepare colony filters for probing with radioactively
labeled probes, ten 96-well plates of the library were thawed at
35   25°C (bench top at room temperature). A replica plating tool with
96 prongs was used to inoculate a fresh 96-well copy plate
containing 75 µl/well of $TB-Amp_{100}$. The copy plate was grown
overnight (stationary) at 37°C, then shaken about 30 min at 100 rpm
at 37°C. A total of 800 colonies was represented in these copy
40   plates, due to nongrowth of some isolates. The replica tool was
used to inoculate duplicate impressions of the 96-well arrays onto
Magna NT (MSI, Westboro, MA) nylon membranes (0.45 micron, 220 x

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250 mm) which had been placed on solid LB-Amp$_{100}$ (100 ml/dish) in
Bio-assay plastic dishes (Nunc, 243 x 243 x 18 mm; Curtin Mathison
Scientific, Inc., Wood Dale, IL). The colonies were grown on the
membranes at 37°C for about 3 hr.

5   A positive control colony (a bacterial clone containing a GZ4
sequence insert, see below) was grown on a separate Magna NT
membrane (Nunc, 0.45 micron, 82 mm circle) on LB medium
supplemented with 35 mg/l chloramphenicol (i.e., LB-Cam$_{35}$), and
processed alongside the library colony membranes. Bacterial
10   colonies on the membranes were lysed, and the DNA was denatured and
neutralized according to a protocol taken from the Genius™ System
User's Guide version 2.0 (Boehringer Mannheim, Indianapolis, IN).
Membranes were placed colony side up on filter paper soaked with
0.5 N NaOH plus 1.5 M NaCl for 15 min to denature, and neutralized
15   on filter paper soaked with 1 M Tris-HCl pH 8.0, 1.5 M NaCl for 15
min. After UV-crosslinking using a Stratagene UV Stratalinker set
on auto crosslink, the membranes were stored dry at 25°C until use.
Membranes were trimmed into strips containing the duplicate
impressions of a single 96-well plate, then washed extensively by
20   the method of section 6.4.1 in CPMB (*op. cit.*): 3 hr at 25°C in 3X
SSC, 0.1% (w/v) SDS, followed by 1 hr at 65°C in the same solution,
then rinsed in 2X SSC in preparation for the hybridization step
(20X SSC = 3 M NaCl, 0.3 M sodium citrate, pH 7.0).

25   <u>Amplification of a Specific Genomic Fragment of a *TcaC* Gene</u>
           Based on the N-terminal amino acid sequence determined for the
purified TcaC peptide fraction [disclosed herein as SEQ ID NO:2], a
pool of degenerate oligonucleotides (pool S4Psh) was synthesized by
standard β-cyanoethyl chemistry on an Applied BioSystem ABI394
30   DNA/RNA Synthesizer (Perkin Elmer, Foster City, CA). The
oligonucleotides were deprotected 8 hours at 55°C, dissolved in
water, quantitated by spectrophotometric measurement, and diluted
for use. This pool corresponds to the determined N-terminal amino
acid sequence of the TcaC peptide. The determined amino acid
35   sequence and the corresponding degenerate DNA sequence are given
below, where A, C, G, and T are the standard DNA bases, and I
represents inosine:

Amino Acid	Met	Gln	Asp	Ser		Pro	Glu	Val

40

S4Psh	5' ATG	CA(A/G)	GA(T/C)	(T/A)(C/G)(T/A)	CCI	GA(A/G)	GT 3'

           Another set of degenerate oligonucleotides was synthesized
(pool P2.3.5R), representing the complement of the coding strand
45   for the determined amino acid sequence of the SEQ ID NO:17:

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Amino Acid	Ala	Phe	Asn	Ile	Asp	Asp	Val
Codons P2.3.5R	5' GCN 3'CG(A/C/G/T)	TT(T/C) AA(A/G)	AA(T/C) TT(A/G)	AT(A/T/C) TA(T/A/G)	GA(T/C) CT(A/G)	GA(T/C) CT(A/G)	GT 3' CA 5'

These oligonucleotides were used as primers in Polymerase Chain Reactions (PCR®, Roche Molecular Systems, Branchburg, NJ) to amplify a specific DNA fragment from genomic DNA prepared from *Photorhabdus* strain W-14 (see above). A typical reaction (50 µl) contained 125 pmol of each primer pool P2Psh and P2.3.5R, 253 ng of genomic template DNA, 10 nmol each of dATP, dCTP, dGTP, and dTTP, 1X GeneAmp® PCR buffer, and 2.5 units of AmpliTaq® DNA polymerase (both from Roche Molecular Systems; 10X GeneAmp® buffer is 100 mM Tris-HCl pH 8.3, 500 mM KCl, 0.01% w/v gelatin). Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler (Perkin Elmer, Foster City, CA) using 35 cycles of 94°C (1.0 min), 55°C (2.0 min), 72°C (3.0 min), followed by an extension period of 7.0 min at 72°C. Amplification products were analyzed by electrophoresis through 2% w/v NuSieve® 3:1 agarose (FMC BioProducts) in TEA buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0). A specific product of estimated size 250 bp was observed amongst numerous other amplification products by ethidium bromide (0.5 µg/ml) staining of the gel and examination under ultraviolet light.

The region of the gel containing an approximately 250 bp product was excised, and a small plug (0.5 mm dia.) was removed and used to supply template for PCR amplification (40 cycles). The reaction (50 µl) contained the same components as above, minus genomic template DNA. Following amplification, the ends of the fragments were made blunt and were phosphorylated by incubation at 25°C for 20 min with 1 unit of T4 DNA polymerase (NEB), 1 nmol ATP, and 2.15 units of T4 kinase (Pharmacia Biotech Inc., Piscataway, NJ).

DNA fragments were separated from residual primers by electrophoresis through 1% w/v GTG® agarose (FMC) in TEA. A gel slice containing fragments of apparent size 250 bp was excised, and the DNA was extracted using a Qiaex kit (Qiagen Inc., Chatsworth, CA).

The extracted DNA fragments were ligated to plasmid vector pBC KS(+) (Stratagene) that had been digested to completion with restriction enzyme *Sma* 1 and extracted in a manner similar to that described for pWE15 DNA above. A typical ligation reaction (16.3 µl) contained 100 ng of digested pBC KS(+) DNA, 70 ng of 250 bp fragment DNA, 1 nmol [Co(NH₃)₆]Cl₃, and 3.9 Weiss units of T4 DNA ligase (Collaborative Biomedical Products, Bedford, MA), in 1X

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ligation buffer (50 mM Tris-HCl, pH .7.4; 10 mM MgCl₂; 10 mM dithiothreitol; 1 mM spermidine, 1 mM ATP, 100 mg/ml bovine serum albumin). Following overnight incubation at 14°C, the ligated products were transformed into frozen, competent *Escherichia coli*

5   DH5α cells (Gibco BRL) according to the suppliers' recommendations, and plated on LB-Cam₃₅ plates, containing IPTG (119 μg/ml) and *X-gal* (50 μg/ml). Independent white colonies were picked, and plasmid DNA was prepared by a modified alkaline-lysis/PEG precipitation method (PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle

10   Sequencing Kit Protocols; ABI/Perkin Elmer). The nucleotide sequence of both strands of the insert DNA was determined, using T7 primers [pBC KS(+) bases 601-623: TAAAACGACGGCCAGTGAGCGCG) and LacZ primers [pBC KS(+) bases 792-816: ATGACCATGATTACGCCAAGCGCGC) and protocols supplied with the PRISM™ sequencing kit (ABI/Perkin

15   Elmer). Nonincorporated dye-terminator dideoxyribonucleotides were removed by passage through Centri-Sep 100 columns (Princeton Separations, Inc., Adelphia, NJ) according to the manufacturer's instructions. The DNA sequence was obtained by analysis of the samples on an ABI Model 373A DNA Sequencer (ABI/Perkin Elmer). The

20   DNA sequences of two isolates, GZ4 and HB14, were found to be as illustrated in Fig. 1.

        This sequence illustrates the following features: 1) bases 1-20 represent one of the 64 possible sequences of the S4Psh degenerate oligonucleotides, ii) the sequence of amino acids 1-3

25   and 6-12 correspond exactly to that determined for the N-terminus of TcaC (disclosed as SEQ ID NO:2), iii) the fourth amino acid encoded is a cysteine residue rather than serine. This difference is encoded within the degeneracy for the serine codons (see above), iv) the fifth amino acid encoded is proline, corresponding to the

30   TcaC N-terminal sequence given as SEQ ID NO:2, v) bases 257-276 encode one of the 192 possible sequences designed into the degenerate pool, vi) the TGA termination codon introduced at bases 268-270 is the result of complementarity to the degeneracy built into the oligonucleotide pool at the corresponding position, and

35   does not indicate a shortened reading frame for the corresponding gene.

Labeling of a TcaC Peptide Gene-specific Probe

        DNA fragments corresponding to the above 276 bases were

40   amplified (35 cycles) by PCR° in a 100 μl reaction volume, using 100 pmol each of P2Psh and P2.3.5R primers, 10 ng of plasmids GZ4 or HB14 as templates, 20 nmol each of dATP, dCTP, dGTP, and dTTP, 5

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units of AmpliTAq° DNA polymerase, and 1X concentration of GeneAmp°
buffer, under the same temperature regimes as described above. The
amplification products were extracted from a 1% GTG° agarose gel by
Qiaex kit and quantitated by fluorometry.

5   The extracted amplification products from plasmid HB14
template (approximately 400 ng) were split into five aliquots and
labeled with ^{32}P-dCTP using the High Prime Labeling Mix (Boehringer
Mannheim) according to the manufacturer's instructions.
Nonincorporated radioisotope was removed by passage through NucTrap°
10   Probe Purification Columns (Stratagene), according to the
supplier's instructions. The specific activity of the labeled DNA
product was determined by scintillation counting to be 3.11 x 10^8
dpm/µg. This labeled DNA was used to probe membranes prepared from
800 members of the genomic library.

15

Screening with a TcaC-peptide Gene Specific Probe

          The radiolabeled HB14 probe was boiled approximately 10 min,
then added to "minimal hyb" solution. [Note: The "minimal hyb"
method is taken from a CERES protocol; "Restriction Fragment Length
20   Polymorphism Laboratory Manual version 4.0", sections 4-40 and 4-
47; CERES/NPI, Salt Lake City, UT. NPI is now defunct, with its
successors operating as Linkage Genetics]. "Minimal hyb" solution
contains 10% w/v PEG (polyethylene glycol, M.W. approx. 8000), 7%
w/v SDS; 0.6X SSC, 10 mM sodium phosphate buffer (from a 1M stock
25   containing 95 g/l $NaH_2PO_4·1H_2O$ and 84.5 g/l $Na_2HPO_4·7H_2O$), 5 mM EDTA,

and 100 mg/ml denatured salmon sperm DNA. Membranes were blotted
dry briefly then, without prehybridization, 5 strips of membrane
were placed in each of 2 plastic boxes containing 75 ml of "minimal
hyb" and 2.6 ng/ml of radiolabeled HB14 probe. These were
30   incubated overnight with slow shaking (50 rpm) at 60°C. The
filters were washed three times for approximately 10 min each at
25°C in "minimal hyb wash solution" (0.25X SSC, 0.2% SDS), followed
by two 30-min washes with slow shaking at 60°C in the same
solution. The filters were placed on paper covered with Saran Wrap°
35   (Dow Brands, Indianapolis, IN) in a light-tight autoradiographic
cassette and exposed to X-Omat X-ray film (Kodak, Rochester, NY)
with two DuPont Cronex Lightning-Plus C1 enhancers (Sigma Chemical
Co., St. Louis, MO), for 4 hr at -70°C. Upon development (standard
photographic procedures), significant signals were evident in both
40   replicates amongst a high background of weaker, more irregular
signals. The filters were again washed for about 4 hr at 68°C in
"minimal hyb wash solution" and then placed again in the cassettes

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and film was exposed overnight at -70°C. Twelve possible positives were identified due to strong signals on both of the duplicate 96-well colony impressions. No signal was seen with negative control membranes (colonies of XL1 Blue MR cells containing pWE15), and a

5   very strong signal was seen with positive control membranes (DH5α cells containing the GZ4 isolate of the PCR product) that had been processed concurrently with the experimental samples.

     The twelve putative hybridization-positive colonies were retrieved from the frozen 96-well library plates and grown

10   overnight at 37°C on solid LB-Amp$_{100}$ medium. They were then patched (3/plate, plus three negative controls: XL1 Blue MR cells containing the pWE15 vector) onto solid LB-Amp$_{100}$. Two sets of membranes (Magna NT nylon, 0.45 micron) were prepared for hybridization. The first set was prepared by placing a filter

15   directly onto the colonies on a patch plate, then removing it with adherent bacterial cells, and processing as below. Filters of the second set were placed on plates containing LB-Amp$_{100}$ medium, then inoculated by transferring cells from the patch plates onto the filters. After overnight growth at 37°C, the filters were removed

20   from the plates and processed.

     Bacterial cells on the filters were lysed and DNA denatured by placing each filter colony-side-up on a pool (1.0 ml) of 0.5 N NaOH in a plastic plate for 3 min. The filters were blotted dry on a paper towel, then the process was repeated with fresh 0.5 N NaOH.

25   After blotting dry, the filters were neutralized by placing each on a 1.0 ml pool of 1 M Tris-HCl, pH 7.5 for 3 min, blotted dry, and reneutralised with fresh buffer. This was followed by two similar soakings (5 min each) on pools of 0.5 M Tris-HCl pH 7.5 plus 1.5 M NaCl. After blotting dry, the DNA was UV crosslinked to the filter

30   (as above), and the filters were washed (25°C, 100 rpm) in about 100 ml of 3X SSC plus 0.1%(w/v) SDS (4 times, 30 min each with fresh solution for each wash). They were then placed in a minimal volume of prehybridization solution [6X SSC plus 1% w/v each of Ficoll 400 (Pharmacia), polyvinylpyrrolidone (av. M.W. 360,000;

35   Sigma) and bovine serum albumin Fraction V; (Sigma)] for 2 hr at 65°C, 50 rpm. The prehybridization solution was removed, and replaced with the HB14 ^{32}P-labeled probe that had been saved from the previous hybridization of the library membranes and which had been denatured at 95°C for 5 min. Hybridization was performed at

40   60°C for 16 hr with shaking at 50 rpm.

     Following removal of the labeled probe solution, the membranes were washed 3 times at 25°C (50 rpm, 15 min) in 3X SSC (about 150 ml each wash). They were then washed for 3 hr at 68°C (50 rpm) in

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0.25X SSC plus 0.2% SDS (minimal hyb wash solution), and exposed to
X-ray film as described above for 1.5 hr at 25°C (no enhancer
screens). This exposure revealed very strong hybridization signals
to cosmid isolates 22G12, 25A10, 26A5, and 26B10, and a very weak

5 signal with cosmid isolate 8B10. No signal was seen with the
negative control (pWE15) colonies, and a very strong signal was
seen with positive control membranes (DH5α cells containing the GZ4
isolate of the PCR product) that had been processed concurrently
with the experimental samples.

10

Amplification of a Specific Genomic Fragment of a TcaB Gene
        Based on the N-terminal amino acid sequence determined for the
purified TcaB$_i$ peptide fraction (disclosed here as SEQ ID NO:3) a
pool of degenerate oligonucleotides (pool P8F) was synthesized as

15 described for peptide TcaC. The determined amino acid sequence and
the corresponding degenerate DNA sequence are given below, where A,
C, G, and T are the standard DNA bases, and I represents inosine:

Amino
20 Acid   Leu   Phe Thr Gln   Thr Leu   Lys Glu Ala Arg

    P8F   5'   TTT ACI CA(A/G) ACI (C/T)TI AAA GAA GCI (A/C)G 3'
            (C/T)TI

25   Another set of degenerate oligonucleotides was synthesized
(pool P8.108.3R), representing the complement of the coding strand
for the determined amino acid sequence of the TcaB$_i$-PT108 internal
peptide (disclosed herein as SEQ ID NO:20):

30 Amino
    Acid   Met Tyr   Tyr   Ile   Gln   Ala   Gln   Gln

    Codons   ATG TA(T/C) TA(T/C) AT(T/C/A) CA(A/G) GC(A/C/G/T) CA(A/G   CA(A/G)
    P8.108.3R   3' AT(A/G) AT(A/G) TA(A/G/T) GT(T/C) CGI   GT(T/C) GT 5'
35   TAC

        These oligonucleotides were used as primers for PCR° using
HotStart 50 Tubes™ (Molecular Bio-Products, Inc., San Diego, CA) to
amplify a specific DNA fragment from genomic DNA prepared from

40 Photorhabdus strain W-14 (see above). A typical reaction (50 µl)
contained (bottom layer) 25 pmol of each primer pool P8F and
P8.108.3R, with 2 nmol each of dATP, dCTP, dGTP, and dTTP, in 1X
GeneAmp° PCR buffer, and (top layer) 230 ng of genomic template DNA,
8 nmol each of dATP, dCTP, dGTP, and dTTP, and 2.5 units of

45 AmpliTaq° DNA polymerase, in 1X GeneAmp° PCR buffer. Amplifications
were performed by 35 cycles as described for the TcaC peptide.
Amplification products were analyzed by electrophoresis through

0.7% w/v SeaKem° LE agarose (FMC) in TEA buffer. A specific product
of estimated size 1600 bp was observed.

Four such reactions were pooled, and the amplified DNA was
extracted from a 1.0% SeaKem° LE gel by Qiaex kit as described for
5   the TcaC peptide. The extracted DNA was used directly as the
template for sequence determination (PRISM™ Sequencing Kit) using
the P8F and P8.108.3R primer pools. Each reaction contained about
100 ng template DNA and 25 pmol of one primer pool, and was
processed according to standard protocols as described for the TcaC
10   peptide. An analysis of the sequence derived from extension of the
P8F primers revealed the short DNA sequence (and encoded amino acid
sequence):

         GAT GCA TTG NTT GCT
         Asp Ala Leu (Val) Ala

15   which corresponds to a portion of the N-terminal peptide sequence
disclosed as SEQ ID NO:3 (TcaB$_i$).

Labeling of a TcaB$_i$-peptide Gene-specific Probe

         Approximately 50 ng of gel-purified TcaB$_i$ DNA fragment was
20   labeled with ^{32}P-dCTP as described above, and nonincorporated
radioisotopes were removed by passage through a NICK Column°
(Pharmacia). The specific activity of the labelled DNA was
determined to be 6 x 10^9 dpm/μg. This labeled DNA was used to probe
colony membranes prepared from members of the genomic library that
25   had hybridized to the TcaC-peptide specific probe.

         The membranes containing the 12 colonies identified in the
TcaC-probe library screen (see above) were stripped of radioactive
TcaC-specific label by boiling twice for approximately 30 min each
time in 1 liter of 0.1X SSC plus 0.1 % SDS. Removal of radiolabel
30   was checked with a 6 hr film exposure. The stripped membranes were
then incubated with the TcaB$_i$ peptide-specific probe prepared
above. The labeled DNA was denatured by boiling for 10 min, and
then added to the filters that had been incubated for 1 hr in 100
ml of "minimal hyb" solution at 60°C. After overnight
35   hybridization at this temperature, the probe solution was removed,
and the filters were washed as follows (all in 0.3X SSC plus 0.1%
SDS): once for 5 min at 25°C, once for 1 hr at 60°C in fresh
solution, and once for 1 hr at 63°C in fresh solution. After 1.5
hr exposure to X-ray film by standard procedures, 4 strongly-
40   hybridizing colonies were observed. These were, as with the TcaC-
specific probe, isolates 22G12, 25A10, 26A5, and 26B10.

The same TcaB$_i$ probe solution was diluted with an equal volume
(about 100 ml) of "minimal hyb" solution, and then used to screen
the membranes containing the 800 members of the genomic library.
After hybridization, washing, and exposure to X-ray film as
5   described above, only the four cosmid clones 22G12, 25A10, 26A5,
and 26B10, were found to hybridize strongly to this probe.

Isolation of Subclones Containing Genes Encoding TcaC and YcaB$_i$
Peptides, and Determination of DNA Base Sequence Thereof

10   Three hybridization-positive cosmids in strain XL1 Blue MR
were grown with shaking overnight (200 rpm) at 30°C in 100 ml TB-
Amp$_{100}$. After harvesting the cells by centrifugation, cosmid DNA
was prepared using a commercially available kit (BIGprep™, 5 Prime
3 Prime, Inc., Boulder, CO), following the manufacturer's
15   protocols. Only one cosmid, 26A5, was successfully isolated by
this procedure. When digested with restriction enzyme *EcoR 1* (NEB)
and analyzed by gel electrophoresis, fragments of approximate sizes
14, 10, 8 (vector), 5, 3.3, 2.9, and 1.5 kbp were detected. A
second attempt to isolate cosmid DNA from the same three strains (8
20   ml cultures; TB-Amp$_{100}$, 30°C) utilized a boiling miniprep method
(Evans G. and G. Wahl., 1987, "Cosmid vectors for genomic walking
and rapid restriction mapping." in *Guide to Molecular Cloning*
Techniques. Meth. Enzymology, Vol. 152, S. Berger and A. Kimmel,
eds., pgs. 604-610). Only one cosmid, 25A10, was successfully
25   isolated by this method. When digested with restriction enzyme
EcoR I (NEB) and analyzed by gel electrophoresis, this cosmid
showed a fragmentation pattern identical to that previously seen
with cosmid 26A5.

A 0.15 µg sample of 26A5 cosmid DNA was used to transform 50
30   ml of *E. coli* DH5α cells (Gibco BRL), by the supplier's protocols.
A single colony isolate of that strain was inoculated into 4 ml of
TB-Amp$_{100}$, and grown for 8 hr at 37°C. Chloramphenicol was added to
a final concentration of 225 µg/ml, incubation was continued for
another 24 hr, then cells were harvested by centrifugation and
35   frozen at -20°C. Isolation of the 26A5 cosmid DNA was by a
standard alkaline lysis miniprep (Maniatis et al., op. cit.,
p. 382), modified by increasing all volumes by 50% and with
stirring or gentle mixing, rather than vortexing, at every step.
After washing the DNA pellet in 70% ethanol, it was dissolved in TE
40   containing 25 µg/ml ribonuclease A (Boehringer Mannheim).

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Identification of *EcoR I* Fragments Hybridizing to GZ4-derived and TcaB_i - Probes

Approximately 0.4 µg of cosmid 25A10 (from XL1 Blue MR cells) and about 0.5 µg of cosmid 26A5 (from chloramphenicol-amplified
5   DH5α cells) were each digested with about 15 units of *EcoR I*(NEB) for 85 min, frozen overnight, then heated at 65°C for five min, and electrophoresed in a 0.7% agarose gel (Seakem° LE, 1X TEA, 80 volts, 90 min). The DNA was stained with ethidium bromide as described above, and photographed under ultraviolet light. The *EcoR I* digest
10 of cosmid 25A10 was a complete digestion, but the sample of cosmid 26A5 was only partially digested under these conditions. The agarose gel containing the DNA fragments was subjected to depurination, denaturation and neutralization, followed by Southern blotting onto a Magna NT nylon membrane, using a high salt (20X
15 SSC) protocol, all as described in section 2.9 of Ausubel *et al.* (CPMB, *op. cit.*). The transferred DNA was then UV-crosslinked to the nylon membrane as before.

An TcaC-peptide specific DNA fragment corresponding to the insert of plasmid isolate GZ4 was amplified by PCR° in a 100 ml
20 reaction volume as described previously above. The amplification products from three such reactions were pooled and were extracted from a 1% GTG° agarose gel by Qiaex kit, as described above, and quantitated by fluorometry. The gel-purified DNA (100 ng) was labeled with ^{32}P-dCTP using the High Prime Labeling Mix (Boehringer
25 Mannheim) as described above, to a specific activity of 6.34 x 10^8 dpm/µg.

The ^{32}P-labeled GZ4 probe was boiled 10 min, then added to "minimal hyb" buffer (at 1 ng/ml), and the Southern blot membrane containing the digested cosmid DNA fragments was added, and
30 incubated for 4 hr at 60°C with gentle shaking at 50 rpm. The membrane was then washed 3 times at 25°C for about 5 min each (minimal hyb wash solution), followed by two washes for 30 min each at 60°C. The blot was exposed to film (with enhancer screens) for about 30 min at -70°C. The GZ4 probe hybridized strongly to the
35 5.0 kbp (apparent size) *EcoR I* fragment of both these two cosmids, 26A5 and 25A10.

The membrane was stripped of radioactivity by boiling for about 30 min in 0.1X SSC plus 0.1 % SDS, and absence of radiolabel was checked by exposure to film. It was then hybridized at 60°C
40 for 3.5 hours with the (denatured) TcaB_i probe in "minimal hyb" buffer previously used for screening the colony membranes (above), washed as described previously, and exposed to film for 40 min at -

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70°C with two enhancer screens. With both cosmids, the TcaB$_i$ probe hybridized lightly with the about 5.0 kbp *EcoR 1* fragment, and strongly with a fragment of approximately 2.9 kbp.

The sample of cosmid 26A5 DNA previously described, (from DH5α
5   cells) was used as the source of DNA from which to subclone the bands of interest. This DNA (2.5 µg) was digested with about 3 units of *EcoR I* (NEB) in a total volume of 30 µl for 1.5 hr, to give a partial digest, as confirmed by gel electrophoresis. Ten µg of pBC KS (+) DNA (Stratagene) were digested for 1.5 hr with 20
10 units of *EcoR I* in a total volume of 20 µl, leading to total digestion as confirmed by electrophoresis. Both *EcoR I-cut* DNA preparations were diluted to 50 µl with water, to each an equal volume of PCI was added, the suspension was gently mixed, spun in a microcentrifuge and the aqueous supernatant was collected. DNA was
15 precipitated by 150 µl ethanol, and the mixture was placed at -20°C overnight. Following centrifugation and drying, the *EcoR I* - digested pBC KS (+) was dissolved in 100 µl TE; the partially digested 26A5 was dissolved in 20 µl TE. DNA recovery was checked by fluorometry.
20   In separate reactions, approximately 60 ng of *EcoR I* -digested pBC KS(+) DNA was ligated with approximately 180 ng or 270 ng of partially digested cosmid 26A5 DNA. Ligations were carried out in a volume of 20 µl at 15°C for 5 hr, using T4 ligase and buffer from New England BioLabs. The ligation mixture, diluted to 100 µl with
25 sterile TE, was used to transform frozen, competent DH5α cells (Gibco BRL) according to the supplier's instructions. Varying amounts (25-200 µl) of the transformed cells were plated on freshly prepared solid LB-Cam$_{35}$ medium with 1 mM IPTG and 50 mg/l X-gal. Plates were incubated at 37°C about 20 hr, then chilled in the dark
30 for approximately 3 hr to intensify color for insert selection. White colonies were picked onto patch plates of the same composition and incubated overnight at 37°C.

Two colony lifts of each of the selected patch plates were prepared as follows. After picking white colonies to fresh plates,
35 round Magna NT nylon membranes were pressed onto the patch plates, the membrane was lifted off, and subjected to denaturation, neutralization and UV crosslinking as described above for the library colony membranes. The crosslinked colony lifts were vigorously washed, including gently wiping off the excess cell
40 debris with a tissue. One set was hybridized with the GZ4(TcaC) probe solution described earlier, and the other set was hybridized with the TcaB$_i$ probe solution described earlier, according to the

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'minimal hyb' protocol, followed by .washing and film exposure as described for the library colony membranes.

Colonies showing hybridization signals either only with the GZ4 probe, with both GZ4 and TcaB$_i$ probes, or only with the TcaB$_i$

5   probe, were selected for further work and cells were streaked for single colony isolation onto LB-Cam$_{35}$ media with IPTG and X-gal as before. Approximately 35 single colonies, from 16 different isolates, were picked into liquid LB-Cam$_{35}$ media and grown overnight at 37°C; the cells were collected by centrifugation and plasmid DNA

10 was isolated by a standard alkaline lysis miniprep according to Maniatis et al. (op. cit. p. 368). DNA pellets were dissolved in TE + 25 µg/ml ribonuclease A and DNA concentration was determined by fluorometry. The EcoR I digestion pattern was analyzed by gel electrophoresis. The following isolates were picked as useful.

15 Isolate A17.2 contains religated pBC KS(+) only and was used for a (negative) control. Isolates D38.3 and C44.1 each contain only the 2.9 kbp, TcaB$_i$ -hybridizing EcoR I fragment inserted into pBC KS(+). These plasmids, named pDAB2000 and pDAB2001, respectively, are illustrated in Fig. 2.

20   Isolate A35.3 contains only the approximately 5 kbp, GZ4)- hybridizing EcoR 1 fragment, inserted into pBC KS(+). This plasmid was named pDAB2002 (also Fig. 2). These isolates provided templates for DNA sequencing.

Plasmids pDAB2000 and pDAB2001 were prepared using the

25 BIGprep™ kit as before. Cultures (30 ml) were grown overnight in TB-Cam$_{35}$ to an OD$_{600}$ of 2, then plasmid was isolated according to the manufacturer's directions. DNA pellets were redissolved in 100 µl TE each, and sample integrity was checked by EcoR I digestion and gel electrophoretic analysis.

30   Sequencing reactions were run in duplicate, with one replicate using as template pDAB2000 DNA, and the other replicate using as template pDAB2001 DNA. The reactions were carried out using the dideoxy dye terminator cycle sequencing method, as described above for the sequencing of the GZ4/HB14 DNAs. Initial sequencing runs

35 utilized as primers the LacZ and T7 primers described above, plus primers based on the determined sequence of the TcaB$_i$ PCR amplification product (TH1 = ATTGCAGACTGCCAATCGCTTCGG, TH12 = GAGAGTATCCAGACCGCGGATGATCTG).

After alignment and editing of each sequencing output, each

40 was truncated to between 250 to 350 bases, depending on the integrity of the chromatographic data as interpreted by the Perkin Elmer Applied Biosystems Division SeqEd 675 software. Subsequent

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sequencing "steps" were made by selecting appropriate sequence for
new primers. With a few exceptions, primers (synthesized as
described above) were 24 bases in length with a 50% G+C
composition. Sequencing by this method was carried out on both
5   strands of the approximately 2.9 kbp EcoR I fragment.

To further serve as template for DNA sequencing, plasmid DNA
from isolate pDAB2002 was prepared by BIGprep™ kit. Sequencing
reactions were performed and analyzed as described above.
Initially, a T3 primer (pBS SK (+) bases 774-796:
10 CGCGCAATTAACCCTCACTAAAG) and a T7 primer (pBS KS (+) bases 621-643:
GCGCGTAATACGACTCACTATAG) were used to prime the sequencing
reactions from the flanking vector sequences, reading into the
insert DNA. Another set of primers; (GZ4F:
GTATCGATTACAACGCTGTCACTTCCC; TH13:   GGGAAGTGACAGCGTTGTAATCGATAC;
15 TH14:   ATGTTGGGTGCGTCGGCTAATGGACATAAC; and LW1-204:
GGGAAGTGACAGCGTTGTAATCGATAC) was made to prime from internal
sequences, which were determined previously by degenerate
oligonucleotide-mediated sequencing of subcloned TcaC-peptide PCR
products. From the data generated during the initial rounds of
20 sequencing, new sets of primers were designed and used to walk the
entire length of the about 5 kbp fragment. A total of 55 oligo
primers was used, enabling the identification of 4832 total bp of
contiguous sequence.

When the DNA sequence of the EcoR I fragment insert of
25 pDAB2002 is combined with part of the determined sequence of the
pDAB2000/pDAB2001 isolates, a total contiguous sequence of 6005 bp
was generated (disclosed herein as SEQ ID NO:25). When long open
reading frames were translated into the corresponding amino acids,
the sequence clearly shows the TcaB$_i$ N-terminal peptide (disclosed
30 as SEQ ID NO:3), encoded by bases 68-124, immediately following a
methionine residue (start of translation). Upstream lies a
potential ribosome binding site (bases 51-58), and downstream, at
bases 215-277 is encoded the TcaB$_i$-PT158 internal peptide
(disclosed herein as SEQ ID NO:19). Further downstream, in the
35 same reading frame, at bases 1787-1822, exists a sequence encoding
the TcaB$_i$-PT108 internal peptide (disclosed herein as SEQ ID
NO:20). Also in the same reading frame, at bases 1946-1972, is
encoded the TcaB$_{ii}$ N-terminal peptide (disclosed herein as SEQ ID
NO:5), and the reading frame continues uninterrupted to a
40 translation termination codon at nucleotides 3632-3634.

The lack of an in-frame stop codon between the end of the
sequence encoding TcaB$_i$ -PT108 and the start of the TcaB$_{ii}$ encoding

region, and the lack of a discernible ribosome binding site immediately upstream of the TcaB$_{ii}$ coding region, indicate that peptides TcaB$_{ii}$ and TcaB$_i$ are encoded by a single open reading frame of 3567 bp beginning at base pair 65 in SEQ ID NO:25), and

5 are most likely derived from a single primary gene product TcaB of 1189 amino acids (131,586 Daltons; disclosed herein as SEQ ID NO:26) by post-translational cleavage. If the amino acid immediately preceding the TcaB$_{ii}$ N-terminal peptide represents the C-terminal amino acid of peptide TcaB$_i$, then the predicted mass of

10 TcaB$_{ii}$ (627 amino acids) is 70,814 Daltons (disclosed herein as SEQ ID NO:28), somewhat higher than the size observed by SDS-PAGE (68 kDa). This peptide would be encoded by a contiguous stretch of 1881 base pairs (disclosed herein as SEQ ID NO:27). It is thought that the native C-terminus of TcaB$_i$ lies somewhat closer to the C-

15 terminus of TcaB$_i$-PT108. The molecular mass of PT108 [3.438 kDa; determined during N-terminal amino acid sequence analysis of this peptide] predicts a size of 30 amino acids. Using the size of this peptide to designate the C-terminus of the TcaB$_i$ coding region [Glu at position 604 of SEQ ID NO:28], the derived size of TcaB$_i$ is

20 determined to be 604 amino acids or 68,463 Daltons, more in agreement with experimental observations.

Translation of the TcaB$_{ii}$ peptide coding region of 1686 base pairs (disclosed herein as SEQ ID NO:29) yields a protein of 562 amino acids (disclosed herein as SEQ ID NO:30) with predicted mass

25 of 60,789 Daltons, which corresponds well with the observed 61 kDa.

A potential ribosome binding site (bases 3682-3687) is found 48 bp downstream of the stop codon for the tcaB open reading frame. At bases 3694-3726 is found a sequence encoding the N-terminus of peptide TcaC, (disclosed as SEQ ID NO:2). The open reading frame

30 initiated by this N-terminal peptide continues uninterrupted to base 6005 (2361 base pairs, disclosed herein as the first 2361 base pairs of SEQ ID NO.31). A gene (tcaC) encoding the entire TcaC peptide, (apparent size about 165 kDa; about 1500 amino acids), would comprise about 4500 bp.

35 Another isolate containing cloned EcoR I fragments of cosmid 26A5, E20.6, was also identified by its homology to the previously mentioned GZ4 and TcaB$_i$ probes. Agarose gel analysis of EcoR I digests of the DNA of the plasmid harbored by this strain (pDAB2004, Fig. 2), revealed insert fragments of estimated sizes

40 2.9, 5, and 3.3 kbp. DNA sequence analysis initiated from primers designed from the sequence of plasmid pDAB2002 revealed that the

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3.3 kbp _EcoR I_ fragment of pDAB2004 lies adjacent to the 5 kbp _EcoR I_ fragment represented in pDAB2002. The 2361 base pair open reading frame discovered in pDAB2002 continues uninterrupted for another 2094 bases in pDAB2004 [disclosed herein as base pairs 2362

5   to 4458 of SEQ ID NO:31]. DNA sequence analysis using the parent cosmid 26A5 DNA as template confirmed the continuity of the open reading frame. Altogether, the open reading frame (_tcaC_ SEQ ID NO:31) comprises 4455 base pairs, and encodes a protein (TcaC) of 1485 amino acids [disclosed herein as SEQ ID NO:32]. The

10   calculated molecular size of 166,214 Daltons is consistent with the estimated size of the TcaC peptide (165 kDa), and the derived amino acid sequence matches exactly that disclosed for the TcaC N-terminal sequence [SEQ ID NO:2].

      The lack of an amino acid sequence corresponding to SEQ ID

15   NO:17; used to design the degenerate oligonucleotide primer pool in the discovered sequence indicates that the generation of the PCR® products found in isolates GZ4 and HB14, which were used as probes in the initial library screen, were fortuitously generated by reverse-strand priming by one of the primers in the degenerate

20   pool. Further, the derived protein sequence does not include the internal fragment disclosed herein as SEQ ID NO:18. These sequences reveal that plasmid pDAB2004 contains the complete coding region for the TcaC peptide.

      Further analysis of SEQ ID NO:25 reveals the end of an open

25   reading frame (bases 1-43), which encodes the final 13 amino acids of the TcaA$_{iii}$ peptide, disclosed herein as SEQ ID NO:35. Only 24 bases separate the end of the TcaA$_{iii}$ coding region and the start of the TcaB$_i$ coding region. Included within the 24 bases are sequences that may serve as a ribosome binding site. Although

30   possible, it is not likely that a _Photorhabdus_ gene promoter is encoded within this short region. We propose that genomic region tca, which includes three long open reading frames [_tcaA_ (SEQ ID NO:33), _tcaB_ (SEQ ID NO:25, bases 65-36334), and _tcaC_ (SEQ ID NO:31),which is separated from the end of _tcaB_ by only 59 bases] is

35   regulated as an operon, with transcription initiating upstream of the start of the _tcaA_ gene (SEQ ID NO:33), and resulting in a polycistronic messenger RNA.

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Example 9

Screening of the *Photorhabdus* Genomic Library
for Genes Encoding the TcbA_ii Peptide

5   This example describes a method used to identify DNA clones
that contain the TcbA_ii peptide-encoding genes, the isolation of
the gene, and the determination of its partial DNA base sequence.

Primers and PCR Reactions

10   The TcbA_ii polypeptide of the insect active preparation is
about 206 kDa. The amino acid sequence of the N-terminus of this
peptide is disclosed as SEQ ID NO:1. Four pools of degenerate
oligonucleotide primers ("Forward primers": TH-4, TH-5, TH-6, and
TH-7) were synthesized to encode a portion of this amino acid
15   sequence, as described in Example 8, and are shown below.

Table 12

Amino Acid	Phe	Ile	Gln	Gly	Tyr	Ser	Asp	Leu	Phe
TH-4	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	TCI	GA(T/C)	CTI	TT-3'
TH-5	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	AG(T/C)	GA(T/C)	CTI	TT-3'
TH-6	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	TCI	GA(T/C)	TT(A/G)	TT-3'
TH-7	5'-TT(T/C)	ATI	CA(A/G)	GGI	TA(T/C)	AG(T/C)	GA(T/C)	TT(A/G)	TT-3'

In addition, a primary ("a") and a secondary ("b") sequence of
30   an internal peptide preparation (TcbA_ii-PT81) have been determined
and are disclosed herein as SEQ ID NO:23 and SEQ ID NO:24,
respectively. Four pools of degenerate oligonucleotides ("Reverse
Primers": TH-8, TH-9, TH-10 and TH-11) were similarly designed and
synthesized to encode the reverse complement of sequences that
35   encode a portion of the peptide of SEQ ID NO:23, as shown below.

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Table 13

Amino Acid	Thr	Tyr	Leu	Thr	Ser	Phe	Glu	Gln	Val	Ala	Asn
TH-8	3'TGI	AT(A/G)	GAI	TGI	AGI	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-9	3'TGI	AT(A/G)	TT(A/G)	TGI	AGI	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-10	3'TGI	AT(A/G)	GAI	TGI	TC(G/A)	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'
TH-11	3'TGI	AT(A/G)	TT(A/G)	TGI	TC(G/A)	AA(A/G)	CT(T/C)	GT(T/C)	CAI	CGI	TT(G/A)-5'

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Sets of these primers were used in PCR° reactions to amplify
TcbA$_{ii}$- encoding gene fragments from the genomic *Photorhabdus*
luminescens W-14 DNA prepared in Example 6. All PCR° reactions were
run with the "Hot Start" technique using AmpliWax™ gems and other

5 Perkin Elmer reagents and protocols. Typically, a mixture (total
volume 11 µl) of MgCl$_2$, dNTP's, 10X GeneAmp° PCR Buffer II, and the
primers were added to tubes containing a single wax bead. [10X
GeneAmp° PCR Buffer II is composed of 100 mM Tris-HCl, pH 8.3; and
500 mM KCl.] The tubes were heated to 80°C for 2 minutes and

10 allowed to cool. To the top of the wax seals, a solution
containing 10X GeneAmp° PCR Buffer II, DNA template, and AmpliTaq°
DNA polymerase were added. Following melting of the wax seal and
mixing of components by thermal cycling, final reaction conditions
(volume of 50 µl) were: 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM

15 MgCl$_2$; 200 µM each in dATP, dCTP, dGTP, dTTP; 1.25 mM in a single
Forward primer pool; 1.25 µM in a single Reverse primer pool, 1.25
units of AmpliTaq° DNA polymerase, and 170 ng of template DNA.

The reactions were placed in a thermocycler (as in Example 8)
and run with the following program:

20

<u>Table 14</u>

Temperature	Time	Cycle Repetition
94°C	2 minutes	1X
94°C	15 seconds	
55-65°C	30 seconds	30X
72°C	1 minute	
72°C	7 minutes	1X
15°C	Constant	

25 A series of amplifications was run at three different
annealing temperatures (55°, 60°, 65°C) using the degenerate primer

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pools. Reactions with annealing at .65°C had no amplification products visible following agarose gel electrophoresis. Reactions having a 60°C annealing regime and containing primers TH-5+TH-10 produced an amplification product that had a mobility corresponding

5   to 2.9 kbp. A lesser amount of the 2.9 kbp product was produced under these conditions with primers TH-7+TH-10. When reactions were annealed at 55°C, these primer pairs produced more of the 2.9 kbp product, and this product was also produced by primer pairs TH-5+TH-8 and TH-5+TH-11. Additional very faint 2.9 kbp bands were

10   seen in lanes containing amplification products from primer pairs TH-7 plus TH-8, TH-9, TH-10, or TH-11.

To obtain sufficient PCR amplification product for cloning and DNA sequence determination, 10 separate PCR reactions were set up using the primers TH-5+TH-10, and were run using the above

15   conditions with a 55°C annealing temperature. All reactions were pooled and the 2.9 kbp product was purified by Qiaex extraction from an agarose gel as described above.

Additional sequences determined for TcbA$_{ii}$ internal peptides are disclosed herein as SEQ ID NO:21 and SEQ ID NO:22. As before,

20   degenerate oligonucleotides (Reverse primers TH-17 and TH-18) were made corresponding to the reverse complement of sequences that encode a portion of the amino acid sequence of these peptides.

Table 15
From SEQ ID NO:21

25

Amino Acid	Met	Glu	Thr	Gln	Asn	Ile	Gln	Glu	Pro
TH-17	3'-TAC	CTT/C	TGI	GTT/C	TTA/G	TAI	GTT/C	GTT/C	GG-5'

30

Table 16
From SEQ ID NO:22

35

Amino Acid	Asn		Pro	Ile	Asn		Ile	Asn		Thr	Gly	Ile	Asp
TH-18	3'-TT(A/G)		GGI	TAI	TT(A/G)		TAI	TT(A?G)		TGI	CCI	TAI	CT(A/G)-5'

40

Degenerate oligonucleotides TH-18 and TH-17 were used in an amplification experiment with *Photorhabdus luminescens* W-14 DNA as template and primers TH-4, TH-5, TH-6, or TH-7 as the 5'- (Forward) primers. These reactions amplified products of approximately 4 kbp

45   and 4.5 kbp, respectively. These DNAs were transferred from agarose gels to nylon membranes and hybridized with a ^{32}P-labeled probe (as described above) prepared from the 2.9 kbp product

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amplified by the TH-5+TH10 primer pair. Both the 4 kbp and the 4.5
kbp amplification products hybridized strongly to the 2.9 kbp
probe. These results were used to construct a map ordering the
TcbA$_{ii}$ internal peptide sequences as shown in Fig. 3. Approximate
5   distances between the primers are shown in nucleotides in Fig. 3.

DNA Sequence of the 2.9 kbp TcbA$_{ii}$-encoding Fragment
        Approximately 200 ng of the purified 2.9 kbp fragment
(prepared above) was precipitated with ethanol and dissolved in 17
10   ml of water. One-half of this was used as sequencing template with
25 pmol of the TH-5 pool as primers, the other half was used as
template for TH-10 priming. Sequencing reactions were as given in
Example 8. No reliable sequence was produced using the TH-10
primer pool; however, reactions with TH-5 primer pool produced the
15   sequence disclosed below:.

```
  1 AATCGTGTTG ATCCCTATGC CGNGCCGGGT TCGGTGGAAT CGATGTCCTC ACCGGGGGTT
 61 TATTNGAGGG ANTNGTCCCG TGAGGCCAAA AANTGGAATG AAAGAAGTTC AATTTNTTAC
121 CTAGATAAAC GTCGCCCGGN TTTAGAAAGN TTANTGNTCA GCCAGAAAAT TTTGGTTGAG
181 GAAATTCCAC CGNTGGTTCT CTCTATTGAT TNGGGCCTGG CCGGGTTCGA ANNAAAACNA
241 GGAAATNCAC AAGTTGAGGT GATGGNTTTG TNGCNANCTT NTCGTTTAGG TGGGGAGAAA
301 CCTTNTCANC ACGNTTNTGA AACTGTCCGG GAAATCGTCC ATGANCGTGA NCCAGGNTTN
361 CGCCATTGG
```

        Based on this sequence, a sequencing primer (TH-21, 5'-
25   CCGGGCGACGTTTATCTAGG-3') was designed to reverse complement bases
120-139, and initiate polymerization towards the 5' end (i.e., TH-5
end) of the gel-purified 2.9 kbp TcbA$_{ii}$-encoding PCR fragment. The
determined sequence is shown below, and is compared to the
biochemically determined N-terminal peptide sequence of TcbA$_{ii}$ SEQ
30   ID NO:1.

TcbA$_{ii}$ 2.9 kbp PCR Fragment Sequence Confirmation
[Underlined amino acids = encoded by degenerate oligonucleotides]

```
35  SEQ ID NO:1   F    I    Q    G    Y    S    D    L    F    G    -    -    A
                  |    |    |    |    |    |    |    |    |    |    |    |
    2.9 kbp seq  GC ATG CAG GGG TAT AGT GAC CTG TTT GGT AAT CGT GCT
                  M    Q    G    Y    S    D    L    F    G    N    R    A >
```

40   From the homology of the derived amino acid sequence to the
biochemically determined one, it is clear that the 2.9 kbp PCR
fragment represents the *TcbA* coding region. This 2.9 kbp fragment
was then used as a hybridization probe to screen the *Photorhabdus*
W-14 genomic library prepared in Example 8 for cosmids containing
45   the TcbA$_{ii}$-encoding gene.

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Screening the _Photorhabdus_ Cosmid Library

     The 2.9 kb gel-purified PCR fragment was labeled with ^{32}P using the Boehringer Mannheim High Prime labeling kit as described in Example 8. Filters containing remnants of approximately 800

5   colonies from the cosmid library were screened as described previously (Example 8), and positive clones were streaked for isolated colonies and rescreened. Three clones (8A11, 25G8, and 26D1) gave positive results through several screening and characterization steps. No hybridization of the TcbA$_{ii}$-specific

10 probe was ever observed with any of the four cosmids identified in Example 8, and which contain the _tcaB_ and _tcaC_ genes. DNA from cosmids 8A11, 25G8, and 26D1 was digested with restriction enzymes _Bgl II_, _EcoR I_ or _Hind III_ (either alone or in combination with one another), and the fragments were separated on an agarose gel and

15 transferred to a nylon membrane as described in Example 8. The membrane was hybridized with ^{32}P-labeled probe prepared from the 4.5 kbp fragment (generated by amplification of _Photorhabdus_ genomic DNA with primers TH-5+TH-17). The patterns generated from cosmid DNAs 8A11 and 26D1 were identical to those generated with

20 similarly-cut genomic DNA on the same membrane. It is concluded that cosmids 8A11 and 26D1 are accurate representations of the genomic TcbA$_{ii}$ encoding locus. However, cosmid 25G8 has a single _Bgl II_ fragment which is slightly larger than the genomic DNA. This may result from positioning of the insert within the vector.

25

DNA Sequence of the _tcbA_-encoding Gene

     The membrane hybridization analysis of cosmid 26D1 revealed that the 4.5 kbp probe hybridized to a single large _EcoR I_ fragment (greater than 9 kbp). This fragment was gel purified and

30 ligated into the _EcoR I_ site of pBC KS (+) as described in Example 8, to generate plasmid pBC-S1/R1. The partial DNA sequence of the insert DNA of this plasmid was determined by "primer walking" from the flanking vector sequence, using procedures described in Example 8. Further sequence was generated by extension from new

35 oligonucleotides designed from the previously determined sequence. When compared to the determined DNA sequence for the _tcbA_ gene identified by other methods (disclosed herein as SEQ ID NO:11 as described in Example 12 below), complete homology was found to nucleotides 1-272, 319-826, 2578-3036, and 3068-3540 (total bases =

40 1712). It was concluded that both approaches can be used to identify DNA fragments encoding the TcbA$_{ii}$ peptide.

Analysis of the Derived Amino Acid Sequence of the tcbA Gene

The sequence of the DNA fragment identified as SEQ ID NO:11 encodes a protein whose derived amino acid sequence is disclosed herein as SEQ ID NO:12. Several features verify the identity of

5   the gene as that encoding the TcbA$_{ii}$ protein. The TcbA$_{ii}$ N-terminal peptide (SEQ ID NO:1; Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala) is encoded as amino acids 88-100. The TcbA$_{ii}$ internal peptide TcbA$_{ii}$-PT81(a) (SEQ ID NO:23) is encoded as amino acids 1065-1077, and TcbA$_{ii}$-PT81(b) (SEQ ID NO:24) is encoded as

10   amino acids 1571-1592. Further, the internal peptide TcbA$_{ii}$-PT56 (SEQ ID NO:22) is encoded as amino acids 1474-1488, and the internal peptide TcbA$_{ii}$-PT103 (SEQ ID NO:21) is encoded as amino acids 1614-1639. It is obvious that this gene is an authentic clone encoding the TcbA$_{ii}$ peptide as isolated from insecticidal

15   protein preparations of Photorhabdus luminescens strain W-14.

The protein isolated as peptide TcbA$_{ii}$ is derived from cleavage of a longer peptide. Evidence for this is provided by the fact that the nucleotides encoding the TcbA$_{ii}$ N-terminal peptide SEQ ID NO:1 are preceded by 261 bases (encoding 87 N-terminal-

20   proximal amino acids) of a longer open reading frame (SEQ ID NO:11). This reading frame begins with nucleotides that encode the amino acid sequence Met Gln Asn Ser Leu, which corresponds to the N-terminal sequence of the large peptide TcbA, and is disclosed herein as SEQ ID NO:16. It is thought that TcbA is the precursor

25   protein for TcbA$_{ii}$.

Relationship of tcbA, tcaB and tcaC Genes

The tcaB and tcaC genes are closely linked and may be transcribed as a single mRNA (Example 8). The tcbA gene is borne

30   on cosmids that apparently do not overlap the ones harboring the tcaB and tcaC cluster, since the respective genomic library screens identified different cosmids. However, comparison of the amino sequences encoded by the tcaB and tcaC genes with the tcbA gene reveals a substantial degree of homology. The amino acid

35   conservation (Protein Alignment Mode of MacVector™ Sequence Analysis Software, scoring matrix pam250, hash value = 2; Oxford Molecular Group, Campbell, CA) is shown in Fig. 4. On the score line of each panel in Fig. 4, up carats (^) indicate homology or conservative amino acid changes,and down carats (v) indicate

40   nonhomology.

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This analysis shows that the amino acid sequence of the TcbA peptide from residues 1739 to 1894 is highly homologous to amino acids 441 to 603 of the TcaB$_i$ peptide (162 of the total 627 amino acids of TcaB; SEQ ID NO:28). In addition, the sequence of TcbA

5   amino acids 1932 to 2459 is highly homologous to amino acids 12 to 531 of peptide TcaB$_{ii}$ (520 of the total 562 amino acids; SEQ ID NO:30). Considering that the TcbA peptide (SEQ ID NO:12) comprises 2505 amino acids, a total of 684 amino acids (27%) at the C-proximal end of it is homologous to the TcaB$_i$ or TcaB$_{ii}$ peptides,

10   and the homologies are arranged colinear to the arrangement of the putative TcaB preprotein (SEQ ID NO:26). A sizeable gap in the TcbA homology coincides with the junction between the TcaB$_i$ and TcaB$_{ii}$ portions of the TcaB preprotein. Clearly the TcbA and TcaB gene products are evolutionarily related, and it is proposed that

15   they share some common function(s) in Photorhabdus.

Example 10

Characterization of Zinc-metalloproteases in Photorhabdus Broth:
Protease Inhibition, Classification, and Purification

20

Protease Inhibition and Classification Assays: Protease assays were performed using FITC-casein dissolved in water as substrate (0.08% final assay concentration). Proteolysis reactions were performed at 25°C for 1 h in the appropriate buffer with 25 μl

25   of Photorhabdus broth (150 μl total reaction volume). Samples were also assayed in the presence and absence of dithiothreitol. After incubation, an equal volume of 12% trichloroacetic acid was added to precipitate undigested protein. Following precipitation for 0.5 h and subsequent centrifugation, 100 μl of the supernatant was

30   placed into a 96-well microtiter plate and the pH of the solution was adjusted by addition of an equal volume of 4N NaOH. Proteolysis was then quantitated using a Fluoroskan II fluorometric plate reader at excitation and emission wavelengths of 485 and 538 nm, respectively. Protease activity was tested over a range from

35   pH 5.0-10.0 in 0.5 units increments. The following buffers were used at 50 mM final concentration: sodium acetate (pH 5.0 - 6.5); Tris-HCL (pH 7.0 - 8.0); and bis-Tris propane (pH 8.5-10.0). To identify the class of protease(s) observed, crude broth was treated with a variety of protease inhibitors (0.5 μg/μl final

40   concentration) and then examined for protease activity at pH 8.0

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using the substrate described above. The protease inhibitors used included E-64 (L-trans-expoxysaccinylleucylamido[4-,-guanidino]-butane), 3,4 dichloroisocoumarin, Leupeptin, pepstatin, amastatin, ethylenediaminetetraacetic acid (EDTA) and 1,10 phenanthroline.

5   Protease assays performed over a pH range revealed that indeed protease(s) were present which exhibited maximal activity at about pH 8.0 (Table 17). Addition of DTT did not have any effect on protease activity. Crude broth was then treated with a variety of protease inhibitors (Table 18). Treatment of crude broth with the
10   inhibitors described above revealed that 1,10 phenanthroline caused complete inhibition of all protease activity when added at a final concentration of 50 μg, with the IC_{50} = 5 μg in 100 μl of a 2 mg/ml crude broth solution. These data indicate that the most abundant protease(s) found in the *Photorhabdus* broth are from the zinc-
15   metalloprotease class of enzymes.

<u>Table 17</u>
<u>Effect of pH on the Protease Activity Found in a Day 1 Production</u>
<u>of *Photorhabdus luminescens* (Strain W-14)</u>

pH	Flu. Units[a]	Percent Activity[b]
5.0	3013 ± 78	17
5.5	7994 ± 448	45
6.0	12965 ± 483	74
6.5	14390 ± 1291	82
7.0	14386 ± 1287	82
7.5	14135 ± 198	80
8.0	17582 ± 831	100
8.5	16183 ± 953	92
9.0	16795 ± 760	96
9.5	16279 ± 1022	93
10.0	15225 ± 210	87

a   Flu. Units = Fluorescence Units (Maximum = about 28,000; background = about 2200).
b   Percent activity relative to the maximum at pH 8.0

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Table 18

Effect of Different Protease Inhibitors on the Protease Activity at
pH 8 Found in a Day 1 Production of *Photorhabdus luminescens*
(Strain W-14)

Inhibitor	Corrected Flu. Units[a]	Percent Inhibition[b]
Control	13053	0
E-64	14259	0
1,10 Phenanthroline[c]	15	99
3,4 Dichloroisocoumarin[d]	7956	39
Leupeptin	13074	0
Pepstatin[c]	13441	0
Amastatin	12474	4
DMSO Control	12005	8
Methanol Control	12125	7

   a Corrected Flu. Units = Fluorescence Units - background (2200
      flu. units).
   b Percent Inhibition relative to protease activity at pH 8.0.
   c Inhibitors were dissolved in methanol.
   d Inhibitors were dissolved in DMSO.

The isolation of a zinc-metalloprotease was performed by
applying dialyzed 10-80% ammonium sulfate pellet to a Q Sepharose
column equilibrated at 50 mM Na_2PO_4, pH 7.0 as described in Example
5 for *Photorhabdus* toxin. After extensive washing, a 0 to 0.5 M
NaCl gradient was used to elute toxin protein. The majority of
biological activity and protein was eluted from 0.15 - 0.45 M NaCl.
However, it was observed that the majority of proteolytic activity
was present in the 0.25-0.35 M NaCl fraction with some activity in
the 0.15-0.25 M NaCl fraction. SDS PAGE analysis of the 0.25-0.35
M NaCl fraction showed a major peptide band of approximately 60
kDa. The 0.15-0.25 M NaCl fraction contained a similar 60 kDa band
but at lower relative protein concentration. Subsequent gel
filtration of this fraction using a Superose 12 HR 16/50 column
resulted in a major peak migrating at 57.5 kDa that contained a
predominant (> 90% of total stained protein) 58.5 kDa band by SDS
PAGE analysis. Additional analysis of this fraction using various
protease inhibitors as described above determined that the protease
was a zinc-metalloprotease. Nearly all of the protease activity
present in *Photorhabdus* broth at day 1 of fermentation corresponded
to the about 58 kDa zinc-metalloprotease.

In yet a second isolation of zinc-metalloprotease(s), W-14
Photorhabdus broth grown for three days was taken and protease
activity was visualized using sodium dodecyl sulfate-polyacrylamide

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gel electrophoresis (SDS-PAGE) laced with gelatin as described in
Schmidt, T.M., Bleakley, B. and Nealson, K.M. 1988. SDS running
gels (5.5 x 8 cm) were made with 12.5 % polyacrylamide (40% stock
solution of acrylamide/bis-acrylamide; Sigma Chemical Co., St.

5   Louis, MO) into which 0.1% gelatin final concentration (Biorad EIA
grade reagent; Richmond CA) was incorporated upon dissolving in
water. SDS-stacking gels (1.0 x 8 cm) were made with 5%
polyacrylamide, also laced with 0.1% gelatin. Typically, 2.5 µg of
protein to be tested was diluted in 0.03 ml of SDS-PAGE loading

10 buffer without dithiothreitol (DTT) and loaded onto the gel.
Proteins were electrophoresed in SDS running buffer (Laemmli, U.K.
1970. Nature 227, 680) at 0° C and at 8 mA. After electrophoresis
was complete, the gel was washed for 2 h in 2.5% (v/v) Triton X-
100. Gels were then incubated for 1 h at 37 °C in 0.1 M glycine

15 (pH 8.0). After incubation, gels were fixed and stained overnight
with 0.1% amido black in methanol-acetic acid- water (30:10:60,
vol./vol./vol.; Sigma Chemical Co.). Protease activity was
visualized as light areas against a dark, amido black stained
background due to proteolysis and subsequent diffusion of

20 incorporated gelatin. At least three distinct bands produced by
proteolytic activity at 58-, 41-, and 38 kDa were observed.

        Activity assays of the different proteases in W-14 day three
culture broth were performed using FITC-casein dissolved in water
as substrate (0.02% final assay concentration). Proteolysis

25 experiments were performed at 37°C for 0-0.5 h in 0.1M Tris-HCl (pH
8.0) with different protein fractions in a total volume of 0.15 ml.
Reactions were terminated by addition of an equal volume of 12%
trichloroacetic acid (TCA) dissolved in water. After incubation at
room temperature for 0.25 h, samples were centrifuged at 10,000 x g

30 for 0.25 h and 0.10 ml aliquots were removed and placed into 96-
well microtiter plates. The solution was then neutralized by the
addition of an equal volume of 2 N sodium hydroxide, followed by
quantitation using a Fluoroskan II fluorometric plate reader with
excitation and emission wavelengths of 485 and 538 nm,

35 respectively. Activity measurements were performed using FITC-
Casein with different protease concentrations at 37°C for 0-10 min.
A unit of activity was arbitrarily defined as the amount of enzyme
needed to produce 1000 fluorescent units/min and specific activity
was defined as units/mg of protease.

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Inhibition studies were performed using two zinc-metalloprotease inhibitors; 1,10 phenanthroline and N-(a-rhamnopyranosyloxyhydroxyphosphinyl)-Leu-Trp(phosphoramidon) with stock solutions of the inhibitors dissolved in 100% ethanol and

5   water, respectively. Stock concentrations were typically 10 mg/ml and 5 mg/ml for 1,10 phenanthroline and phosphoramidon, respectively, with final concentrations of inhibitor at 0.5-1.0 mg/ml per reaction. Treatment of three day W-14 crude broth with 1,10 phenanthroline, an inhibitor of all zinc metalloproteases,

10   resulted in complete elimination of all protease activity while treatment with phosphoramidon, an inhibitor of thermolysin-like proteases (Weaver, L.H., Kester, W.R., and Matthews, B.W. 1977. J. Mol. Biol. 114, 119-132), resulted in about 56% reduction of protease activity. The residual proteolytic activity could not be

15   further reduced with additional phosphoramidon.

The proteases of three day W-14 *Photorhabdus* broth were purified as follows: 4.0 liters of broth were concentrated using an Amicon spiral ultra filtration cartridge Type S1Y100 attached to an Amicon M-12 filtration device. The flow-through material having

20   native proteins less than 100 kDa in size (3.8 L) was concentrated to 0.375 L using an Amicon spiral ultra filtration cartridge Type S1Y10 attached to an Amicon M-12 filtration device. The retentate material contained proteins ranging in size from 10-100 kDa. This material was loaded onto a Pharmacia HR16/10 column which had been

25   packed with PerSeptive Biosystem (Framington, MA) Poros® 50 HQ strong anion exchange packing that had been equilibrated in 10 mM sodium phosphate buffer (pH 7.0). Proteins were loaded on the column at a flow rate of 5 ml/min, followed by washing unbound protein with buffer until A_{280} = 0.00. Afterwards, proteins were

30   eluted using a NaCl gradient of 0-1.0 M NaCl in 40 min at a flow rate of 7.5 ml/min. Fractions were assayed for protease activity, *supra.*, and active fractions were pooled. Proteolytically active fractions were diluted with 50% (v/v) 10 mM sodium phosphate buffer (pH 7.0) and loaded onto a Pharmacia HR 10/10 Mono Q column

35   equilibrated in 10 mM sodium phosphate. After washing the column with buffer until A_{280} = 0.00, proteins were eluted using a NaCl gradient of 0-0.5 M NaCl for 1 h at a flow rate of 2.0 ml/min. Fractions were assayed for protease activity. Those fractions having the greatest amount of phosphoramidon-sensitive protease

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activity, the phosphoramidon sensitive activity being due to the
41/38 kDa protease, *infra.*, were pooled. These fractions were
found to elute at a range of 0.15-0.25 M NaCl. Fractions
containing a predominance of phosphoramidon-insensitive protease
5   activity, the 58 kDa protease, were also pooled. These fractions
were found to elute at a range of 0.25-0.35 M NaCl. The
phosphoramidon-sensitive protease fractions were then concentrated
to a final volume of 0.75 ml using a Millipore Ultrafree®-15
centrifugal filter device Biomax-5K NMWL membrane. This material
10   was applied at a flow rate of 0.5 ml/min to a Pharmacia HR 10/30
column that had been packed with Pharmacia Sephadex G-50
equilibrated in 10 mM sodium phosphate buffer (pH 7.0)/ 0.1 M NaCl.
Fractions having the maximal phosphoramidon-sensitive protease
activity were then pooled and centrifuged over a Millipore
15   Ultrafree®-15 centrifugal filter device Biomax-50K NMWL membrane.
Proteolytic activity analysis, *supra.*, indicated this material to
have only phosphoramidon-sensitive protease activity. Pooling of
the phosphoramidon-insensitive protease, the 58 kDa protein, was
followed by concentrating in a Millipore Ultrafree®-15 centrifugal
20   filter device Biomax-50K NMWL membrane and further separation on a
Pharmacia Superdex-75 column. Fractions containing the protease
were pooled.

Analysis of purified 58- and 41/38 kDa purified proteases
revealed that, while both types of protease were completely
25   inhibited with 1,10 phenanthroline, only the 41/38 kDa protease was
inhibited with phosphoramidon. Further analysis of crude broth
indicated that protease activity of day 1 W-14 broth has 23% of the
total protease activity due to the 41/38 kDa protease, increasing
to 44% in day three W-14 broth.

30   Standard SDS-PAGE analysis for examining protein purity and
obtaining amino terminal sequence was performed using 4-20%
gradient MiniPlus SepraGels purchased from Integrated Separation
Systems (Natick, MA). Proteins to be amino-terminal sequenced were
blotted onto PVDF membrane following purification, *infra.*,
35   (ProBlott™ Membranes; Applied Biosystems, Foster City, CA),
visualized with 0.1% amido black, excised, and sent to Cambridge
Prochem; Cambridge, MA, for sequencing.

Deduced amino terminal sequence of the 58- (SEQ ID NO:45) and
41/38 kDa (SEQ ID NO:44) proteases from three day old W-14 broth

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were DV-GSEKANEKLK (SEQ ID NO: 45) and DSGDDDKVTNTDIHR (SEQ ID
NO:44), respectively.

Sequencing of the 41/38 kDa protease revealed several amino
termini, each one having an additional amino acid removed by
5   proteolysis. Examination of the primary, secondary, tertiary and
quartenary sequences for the 38 and 41 kDa polypeptides allowed for
deduction of the sequence shown above and revealed that these two
proteases are homologous.

10   Example 11, Part A
Screening of Photorhabdus Genomic Library Via Use of Antibodies for
                     Genes Encoding TcbA Peptide

In parallel to the sequencing described above, suitable
15   probing and sequencing was done based on the TcbA$_{ii}$ peptide (SEQ ID
NO:1). This sequencing was performed by preparing bacterial
culture broths and purifying the toxin as described in Examples 1
and 2 above.

Genomic DNA was isolated from the Photorhabdus luminescens
20   strain W-14 grown in Grace's insect tissue culture medium. The
bacteria were grown in 5 ml of culture medium in a 250 ml
Erlenmeyer flask at 28°C and 250 rpm for approximately 24 hours.
Bacterial cells from 100 ml of culture medium were pelleted at 5000
x g for 10 minutes. The supernatant was discarded, and the cell
25   pellets then were used for the genomic DNA isolation.

The genomic DNA was isolated using a modification of the CTAB
method described in Section 2.4.3 of Ausubel (supra.). The section
entitled "Large Scale CsCl prep of bacterial genomic DNA" was
followed through step 6. At this point, an additional
30   chloroform/isoamyl alcohol (24:1) extraction was performed followed
by a phenol/chloroform/isoamyl (25:24:1) extraction step and a
final chloroform/isoamyl/alcohol (24:1) extraction. The DNA was
precipitated by the addition of a 0.6 volume of isopropanol. The
precipitated DNA was hooked and wound around the end of a bent
35   glass rod, dipped briefly into 70% ethanol as a final wash, and
dissolved in 3 ml of TE buffer.

The DNA concentration, estimated by optical density at 280/260
nm, was approximately 2 mg/ml.

Using this genomic DNA, a library was prepared. Approximately
40   50 μg of genomic DNA was partly digested with Sau3 Al. Then NaCl
density gradient centrifugation was used to size fractionate the
partially digested DNA fragments. Fractions containing DNA

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fragments with an average size of 12 kb, or larger, as determined
by agarose gel electrophoresis, were ligated into the plasmid
BluScript, Stratagene, La Jolla, California, and transformed into
an *E. coli* DH5α or DHB10 strain.

5   Separately, purified aliquots of the protein were sent to the
biotechnology hybridoma center at the University of Wisconsin,
Madison for production of monoclonal antibodies to the proteins.
The material that was sent was the HPLC purified fraction
containing native bands 1 and 2 which had been denatured at 65°C,

10   and 20 µg of which was injected into each of four mice. Stable
monoclonal antibody-producing hybridoma cell lines were recovered
after spleen cells from unimmunized mouse were fused with a stable
myeloma cell line. Monoclonal antibodies were recovered from the
hybridomas.

15   Separately, polyclonal antibodies were created by taking
native agarose gel purified band 1 (see Example 1) protein which
was then used to immunize a New Zealand white rabbit. The protein
was prepared by excising the band from the native agarose gels,
briefly heating the gel pieces to 65°C to melt the agarose, and

20   immediately emulsifying with adjuvant. Freund's complete adjuvant
was used for the primary immunizations and Freund's incomplete was
used for 3 additional injections at monthly intervals. For each
injection, approximately 0.2 ml of emulsified band 1, containing 50
to 100 micrograms of protein, was delivered by multiple

25   subcontaneous injections into the back of the rabbit. Serum was
obtained 10 days after the final injection and additional bleeds
were performed at weekly intervals for 3 weeks. The serum
complement was inactivated by heating to 56°C for 15 minutes and
then stored at -20°C.

30   The monoclonal and polyclonal antibodies were then used to
screen the genomic library for the expression of antigens which
could be detected by the epitope. Positive clones were detected on
nitrocellulose filter colony lifts. An immunoblot analysis of the
positive clones was undertaken.

35   An analysis of the clones as defined by both immunoblot and
Southern analysis resulted in the tentative identification of four
genomic regions.

         In the first region was a gene encoding the peptide designated
here as TcbA$_{ii}$. Full DNA sequence of this gene (*tcbA*) was

40   obtained. It is set forth as SEQ ID NO:11. Confirmation that the
sequence encodes the internal sequence of SEQ ID NO:1 is
demonstrated by the presence of SEQ ID NO:1 at amino acid number 88

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from the deduced amino acid sequence created by the open reading
frame of SEQ ID NO:11. This can be confirmed by referring to SEQ
ID NO:12, which is the deduced amino acid sequence created by SEQ
ID NO:11.

5   The second region of toxin peptides contains the segments
referred to above as TcaB$_i$, TcaB$_{ii}$ and TcaC. Following the
screening of the library with the polyclonal antisera, this second
region of toxin genes was identified by several clones which
produced different size proteins, all of which cross-reacted with
10   the polyclonal antibody on an immunoblot and were also found to
share DNA homology on a Southern Blot. Sequence comparison
revealed that they belonged to the gene complex designated *TcaB* and
TcaC above.

         Two other regions of antibody toxin clones were also isolated
15   in the polyclonal screen. These regions produced proteins that
cross-react with a polyclonal antibody and also shared DNA homology
with the regions as determined by Southern blotting. Thus, it
appears that the *Photorhabdus luminescens* extracellular protein
genes represent a family of genes which are evolutionarily related.

20   To further pursue the concept that there might be
evolutionarily related variations in the toxin peptides contained
within this organism, two approaches have been undertaken to
examine other strains of *Photorhabdus luminescens* for the presence
of related proteins. This was done both by PCR amplification of
25   genomic DNA and by immunoblot analysis using the polyclonal and
monoclonal antibodies.

         The results indicate that related proteins are produced by
Photorhabdus. luminescens strains WX-2, WX-3, WX-4, WX-5, WX-6, WX-
7, WX-8, WX-11, WX-12, WX-15 and W-14.

30

Example 11, Part B
Sequence and Analysis of *tcc* Toxin Clones

         Further DNA sequencing was performed on plasmids isolated from
35   *E. coli* clones described in Example 11, Part A. The nucleotide
sequence from the third region of *E. coli* clones was shown to be
three closely linked open reading frames at this genomic locus.
This locus was designated *tcc* with the three open reading frames
designated *tccA* SEQ ID NO:56, *tccB* SEQ ID NO:58 and *tccC* SEQ ID
40   NO:60. The close linkage between these open reading frames is
revealed by examination of SEQ ID NO:56, in which 93 bp separate
the stop codon of *tccA* from the start codon of *tccb* (bases 2992-
2994 of SEQ ID NO:56), and by examination of SEQ ID NO:58, in which

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131 bases separate the stop codon of *tccB* and the *tccC* (bases 4930-4932 of SEQ ID NO:58). The physical map is presented in Fig. 6B.

The deduced amino acid sequence from the *tccA* open reading frame indicates that the gene encodes a protein of 105,459 Da.
5   This protein was designated TccA (SEQ ID NO:57). The first 12 amino acids of this protein match the N-terminal sequence obtained from a 108 kDa protein, SEQ ID NO:8, previously identified as part of the toxin complex.

The deduced amino acid sequence from the *tccB* open reading
10   frame indicates that this gene encodes a protein of 175,716 Da. This protein was designated TccB (SEQ ID NO:59). The first 11 amino acids of this protein match the N-terminal sequence obtained from a protein with estimated molecular weight of 185 kDa, SEQ ID NO:7. Similarity analysis revealed that the TccB protein is related
15   to the proteins identified as TcbA SEQ ID NO:12; 37% similarity and 28% identity, TcdA SEQ ID NO:47; 35% similarity and 28%identity, and TcaB SEQ ID NO:26; 32% similarity and 26% identity (using the GAP algorithm Wisconsin Package Version 9.0, Genetics Computer Group (GCG) Madison Wisconsin).

20   The deduced amino acid sequence of *tccC* indicated that this open reading frame encodes a protein of 111,694 Da and the protein product was designated TccC (SEQ ID NO:61).

Example 12
25   ## Characterization of *Photorhabdus* Strains

In order to establish that the collection described herein was comprised of *Photorhabdus* strains, the strains herein were assessed in terms of recognized microbiological traits that are
30   characteristic of *Photorhabdus* and which differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* spp. (Farmer, J. J. 1984. Bergey's Manual of Systemic Bacteriology, Vol 1. pp. 510-511. (ed. Kreig N. R. and Holt, J. G.). Williams & Wilkins, Baltimore; Akhurst and Boemare, 1988, Boemare et al., 1993). These
35   characteristic traits are as follows: Gram's stain negative rods, organism size of 0.5-2 μm in width and 2-10 μm in length, red/yellow colony pigmentation, presence of crystalline inclusion bodies, presence of catalase, inability to reduce nitrate, presence of bioluminescence, ability to take up dye from growth media,
40   positive for protease production, growth-temperature range below 37°C, survival under anaerobic conditions and positively motile.

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(Table 20). Reference *Escherichia coli*, *Xenorhabdus* and *Photorhabdus* strains were included in all tests for comparison. The overall results are consistent with all strains being part of the family Enterobacteriaceae and the genus *Photorhabdus*.

A luminometer was used to establish the bioluminescence of each strain and provide a quantitative and relative measurement of light production. For measurement of relative light emitting units, the broths from each strain (cells and media) were measured at three time intervals after inoculation in liquid culture (6, 12, and 24 hr) and compared to background luminosity (uninoculated media and water). Prior to measuring light emission from the various broths, cell density was established by measuring light absorbance (560 nM) in a Gilford Systems (Oberlin, OH) spectrophotometer using a sipper cell. Appropriate dilutions were then made (to normalize optical density to 1.0 unit) before measuring luminosity. Aliquots of the diluted broths were then placed into cuvettes (300 µl each) and read in a Bio-Orbit 1251 Luminometer (Bio-Orbit Oy, Twiku, Finland). The integration period for each sample was 45 seconds. The samples were continuously mixed (spun in baffled cuvettes) while being read to provide oxygen availability. A positive test was determined as being ≥ 5-fold background luminescence (about 5-10 units). In addition, colony luminosity was detected with photographic film overlays and visually, after adaptation in a darkroom. The Gram's staining characteristics of each strain were established with a commercial Gram's stain kit (BBL, Cockeysville, MD) used in conjunction with Gram's stain control slides (Fisher Scientific, Pittsburgh, PA). Microscopic evaluation was then performed using a Zeiss microscope (Carl Zeiss, Germany) 100X oil immersion objective lens (with 10X ocular and 2X body magnification). Microscopic examination of individual strains for organism size, cellular description and inclusion bodies (the latter after logarithmic growth) was performed using wet mount slides (10X ocular, 2X body and 40X objective magnification) with oil immersion and phase contrast microscopy with a micrometer (Akhurst, R.J. and Boemare, N.E. 1990. *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. and Kaya, H.). pp. 75-90. CRC Press, Boca Raton, USA.; Baghdiguian S., Boyer-Giglio M.H., Thaler, J.O., Bonnot G., Boemare N. 1993. Biol. Cell 79, 177-185.). Colony pigmentation was observed after

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inoculation on Bacto nutrient agar, (Difco Laboratories, Detroit,
MI) prepared as per label instructions. Incubation occurred at
28°C and descriptions were produced after 5-7 days. To test for
the presence of the enzyme catalase, a colony of the test organism
5   was removed on a small plug from a nutrient agar plate and placed
into the bottom of a glass test tube. One ml of a household
hydrogen peroxide solution was gently added down the side of the
tube. A positive reaction was recorded when bubbles of gas
(presumptive oxygen) appeared immediately or within 5 seconds.
10   Controls of uninoculated nutrient agar and hydrogen peroxide
solution were also examined. To test for nitrate reduction, each
culture was inoculated into 10 ml of Bacto Nitrate Broth (Difco
Laboratories, Detroit, MI). After 24 hours incubation at 28°C,
nitrite production was tested by the addition of two drops of
15   sulfanilic acid reagent and two drops of alpha-naphthylamine
reagent (see Difco Manual, 10th edition, Difco Laboratories,
Detroit, MI, 1984). The generation of a distinct pink or red color
indicates the formation of nitrite from nitrate. The ability of
each strain to uptake dye from growth media was tested with Bacto
20   MacConkey agar containing the dye neutral red; Bacto Tergitol-7
agar containing the dye bromothymol blue and Bacto EMB Agar
containing the dye eosin-Y (agars from Difco Laboratories, Detroit,
MI, all prepared according to label instructions). After
inoculation on these media, dye uptake was recorded after
25   incubation at 28°C for 5 days. Growth on these latter media is
characteristic for members of the family *Enterobacteriaceae*.
Motility of each strain was tested using a solution of Bacto
Motility Test Medium (Difco Laboratories, Detroit, MI) prepared as
per label instructions. A butt-stab inoculation was performed with
30   each strain and motility was judged macroscopically by a diffuse
zone of growth spreading from the line of inoculum. In many cases,
motility was also observed microscopically from liquid culture
under wet mount slides. Biochemical nutrient evaluation for each
strain was performed using BBL Enterotube II (Benton, Dickinson,
35   Germany). Product instructions were followed with the exception
that incubation was carried out at 28°C for 5 days. Results were
consistent with previously cited reports for *Photorhabdus*. The
production of protease was tested by observing hydrolysis of
gelatin using Bacto gelatin (Difco Laboratories, Detroit, MI)

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plates made as per label instructions. Cultures were inoculated and the plates were incubated at 28°C for 5 days. To assess growth at different temperatures, agar plates [2% proteose peptone #3 with two percent Bacto-Agar (Difco, Detroit, MI) in deionized water]

5   were streaked from a common source of inoculum. Plates were sealed with Nesco® film and incubated at 20, 28 and 37°C for up to three weeks. Plates showing no growth at 37°C showed no cell viability after transfer to a 28°C incubator for one week. Oxygen requirements for *Photorhabdus* strains were tested in the following

10 manner. A butt-stab inoculation into fluid thioglycolate broth medium (Difco, Detroit, MI) was made. The tubes were incubated at room temperature for one week and cultures were then examined for type and extent of growth. The indicator resazurin demonstrates the level of medium oxidation or the aerobiosis zone (Difco Manual,

15 10th edition, Difco Laboratories, Detroit, MI). Growth zone results obtained for the *Photorhabdus* strains tested were consistent with those of a facultative anaerobic microorganism.

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Table 19

Taxonomic Traits of *Photorhabdus* Strains

Traits Assessed*

Strain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
W-14	–†	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
WX-1	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
WX-2	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
WX-3	–	±	±	rd S	±	–	±	±	±	YT	±	±	±	±	±	±	–
WX-4	–	±	±	rd S	±	–	±	±	±	YT	±	±	±	±	±	±	–
WX-5	–	±	±	rd S	±	–	±	±	±	LO	±	±	±	±	±	±	–
WX-6	–	±	±	rd S	±	–	±	±	±	LY	±	±	±	±	±	±	–
WX-7	–	±	±	rd S	±	–	±	±	±	R	±	±	±	±	±	±	–
WX-8	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
WX-9	–	±	±	rd S	±	–	±	±	±	YT	±	±	±	±	±	±	–
WX-10	–	±	±	rd S	±	–	±	±	±	RO	±	±	±	±	±	±	–
WX-11	–	±	±	rd S	±	–	±	±	±	RO	±	±	±	±	±	±	–
WX-12	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
WX-14	–	±	±	rd S	±	–	±	±	±	LR	±	±	±	±	±	±	–
WX-15	–	±	±	rd S	±	–	±	±	±	LR	±	±	±	±	±	±	–
H9	–	±	±	rd S	±	–	±	±	±	LY	±	±	±	±	±	±	–
Hb	–	±	±	rd S	±	–	±	±	±	YT	±	±	±	±	±	±	–
Hm	–	±	±	rd S	±	–	±	±	±	TY	±	±	±	±	±	±	–
HP88	–	±	±	rd S	±	–	±	±	±	LY	±	±	±	±	±	±	–
NC-1	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
W30	–	±	±	rd S	±	–	±	±	±	YT	±	±	±	±	±	±	–
WIR	–	±	±	rd S	±	–	±	±	±	RO	±	±	±	±	±	±	–
B2	–	±	±	rd S	±	–	±	±	±	R	±	±	±	±	±	±	–
43948	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
43949	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
43950	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
43951	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–
43952	–	±	±	rd S	±	–	±	±	±	O	±	±	±	±	±	±	–

5 * - A = Gram's stain, B=Crystaline inclusion bodies,
C=Bioluminescence, D=Cell form, E=Motility, F=Nitrate reduction,
G=Presence of catalase, H=Gelatin hydrolysis, I=Dye uptake,
J=Pigmentation, K=Growth on EMB agar, L=Growth on MacConkey agar,
M=Growth on Tergitol-7 agar, N=Facultative anaerobe, O=Growth at
10 20°C, P=Growth at 28°C, Q=Growth at 37°C, † - +/- = positive or
negative for trait, rd=rod, S=sized within Genus descriptors,
RO=red-orange, LR = light red, R= red, O= orange, Y= yellow, T=
tan, LY= light yellow, YT= yellow tan, and LO= light orange.

15   Cellular fatty acid analysis is a recognized tool for

bacterial characterization at the genus and species level

(Tornabene, T. G. 1985. *Lipid Analysis and the Relationship to*

Chemotaxonomy in Methods in Microbiology, Vol. 18, 209-234.;

Goodfellow, M. and O'Donnell, A. G. 1993. *Roots of Bacterial*

20 *Systematics in Handbook of New Bacterial Systematics* (ed.

Goodfellow, M. & O'Donnell, A. G.) pp. 3-54. London: Academic Press

Ltd.), these references are incorporated herein by reference, and

were used to confirm that our collection was related at the genus

level. Cultures were shipped to an external, contract laboratory

for fatty acid methyl ester analysis (FAME) using a Microbial ID
(MIDI, Newark, DE, USA) Microbial Identification System (MIS). The
MIS system consists of a Hewlett Packard HP5890A gas chromatograph
with a 25mm x 0.2mm 5% methylphenyl silicone fused silica capillary

5   column. Hydrogen is used as the carrier gas and a flame-ionization
detector functions in conjunction with an automatic sampler,
integrator and computer. The computer compares the sample fatty
acid methyl esters to a microbial fatty acid library and against a
calibration mix of known fatty acids. As selected by the contract

10   laboratory, strains were grown for 24 hours at 28°C on trypticase
soy agar prior to analysis. Extraction of samples was performed by
the contract lab as per standard FAME methodology. There was no
direct identification of the strains to any luminescent bacterial
group other than *Photorhabdus*. When the cluster analysis was

15   performed, which compares the fatty acid profiles of a group of
isolates, the strain fatty acid profiles were related at the genus
level.

       The evolutionary diversity of the *Photorhabdus* strains in our
collection was measured by analysis of PCR (Polymerase Chain

20   Reaction) mediated genomic fingerprinting using genomic DNA from
each strain. This technique is based on families of repetitive DNA
sequences present throughout the genome of diverse bacterial
species (reviewed by Versalovic, J., Schneider, M., DE Bruijn,
F. J. and Lupski, J. R. 1994. Methods Mol. Cell. Biol., 5, 25-40.).

25   Three of these, repetitive extragenic palindromic sequence (REP),
enterobacterial repetitive intergenic consensus (ERIC) and the BOX
element are thought to play an important role in the organization
of the bacterial genome. Genomic organization is believed to be
shaped by selection and the differential dispersion of these

30   elements within the genome of closely related bacterial strains can
be used to discriminate these strains (e.g., Louws, F. J.,
Fulbright, D. W., Stephens, C. T. and DE Bruijn, F. J. 1994. Appl.
Environ. Micro. 60, 2286-2295). Rep-PCR utilizes oligonucleotide
primers complementary to these repetitive sequences to amplify the

35   variably sized DNA fragments lying between them. The resulting
products are separated by electrophoresis to establish the DNA
"fingerprint" for each strain.

       To isolate genomic DNA from our strains, cell pellets were
resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a

final volume of 10 ml and 12 ml of 5 M NaCl was then added. This
mixture was centrifuged 20 min. at 15,000 x g. The resulting
pellet was resuspended in 5.7 ml of TE and 300 μl of 10% SDS and 60
μl 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY)

5   were added. This mixture was incubated at 37 °C for 1 hr,
approximately 10 mg of lysozyme was then added and the mixture was
incubated for an additional 45 min. One milliliter of 5M NaCl and
800 μl of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl) were then
added and the mixture was incubated 10 min. at 65°C, gently

10   agitated, then incubated and agitated for an additional 20 min. to
aid in clearing of the cellular material. An equal volume of
chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed
gently then centrifuged. Two extractions were then performed with
an equal volume of phenol/chloroform/isoamyl alcohol (50:49:1).

15   Genomic DNA was precipitated with 0.6 volume of isopropanol.
Precipitated DNA was removed with a glass rod, washed twice with
70% ethanol, dried and dissolved in 2 ml of STE (10 mM Tris-HCl
pH8.0, 10 mM NaCl, 1 mM EDTA). The DNA was then quantitated by
optical density at 260 nm. To perform rep-PCR analysis of

20   *Photorhabdus* genomic DNA the following primers were used, REP1R-I;
5'-IIIICGICGICATCIGGC-3' and REP2-I; 5'-ICGICTTATCIGGCCTAC-3'. PCR
was performed using the following 25μl reaction: 7.75 μl H2O, 2.5
μl 10X LA buffer (PanVera Corp., Madison, WI), 16 μl dNTP mix (2.5
mM each), 1 μl of each primer at 50 pM/μl, 1 μl DMSO, 1.5 μl

25   genomic DNA (concentrations ranged from 0.075-0.480 μg/μl) and 0.25
μl TaKaRa EX Taq (PanVera Corp., Madison, WI). The PCR
amplification was performed in a Perkin Elmer DNA Thermal Cycler
(Norwalk, CT) using the following conditions: 95°C/7 min. then 35
cycles of; 94°C/1 min.,44°C/1 min., 65°C/8 min., followed by 15 min.

30   at 65°C. After cycling, the 25 μl reaction was added to 5 μl of 6X
gel loading buffer (0.25% bromophenol blue, 40% w/v sucrose in
H2O). A 15x20cm 1%-agarose gel was then run in TBE buffer (0.09 M
Tris-borate, 0.002 M EDTA) using 8 μl of each reaction. The gel
was run for approximately 16 hours at 45v. Gels were then stained

35   in 20 μg/ml ethidium bromide for 1 hour and destained in TBE buffer
for approximately 3 hours. Polaroid® photographs of the gels were
then taken under UV illumination.

The presence or absence of bands at specific sizes for each
strain was scored from the photographs and entered as a similarity

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matrix in the numerical taxonomy software program, NTSYS-pc (Exeter
Software, Setauket, NY). Controls of *E. coli* strain HB101 and
Xanthomonas oryzae pv. oryzae assayed at the same time produced PCR
"fingerprints" corresponding to published reports (Versalovic, J.,

5   Koeuth, T. and Lupski, J. R. 1991. Nucleic Acids Res. 19, 6823-
6831; Vera Cruz, C. M., Halda-Alija, L., Louws, F., Skinner, D. Z.,
George, M. L., Nelson, R. J., DE Bruijn, F. J., Rice, C. and Leach,
J. E. 1995. Int. Rice Res. Notes, 20, 23-24.; Vera Cruz, C. M.,
Ardales, E. Y., Skinner, D. Z., Talag, J., Nelson, R. J., Louws,

10   F. J., Leung, H., Mew, T. W. and Leach, J. E. 1996. Phytopathology
(in press, respectively). The data from *Photorhabdus* strains were
then analyzed with a series of programs within NTSYS-pc; SIMQUAL
(Similarity for Qualitative data) to generate a matrix of
similarity coefficients (using the Jaccard coefficient) and SAHN

15   (Sequential, Agglomerative, Heirarchical and Nested) clustering
[using the UPGMA (Unweighted Pair-Group Method with Arithmetic
Averages) method] which groups related strains and can be expressed
as a phenogram (Fig. 5). The COPH (cophenetic values) and MXCOMP
(matrix comparison) programs were used to generate a cophenetic

20   value matrix and compare the correlation between this and the
original matrix upon which the clustering was based. A resulting
normalized Mantel statistic (r) was generated which is a measure of
the goodness of fit for a cluster analysis (r=0.8-0.9 represents a
very good fit). In our case r = 0.919. Therefore, our collection

25   is comprised of a diverse group of easily distinguishable strains
representative of the *Photorhabdus* genus.

<center>Example 13</center>
<center>Insecticidal Utility of Toxin(s) Produced</center>
30   <center>by Various *Photorhabdus* Strains</center>

Initial "seed" cultures of the various *Photorhabdus* strains
were produced by inoculating 175 ml of 2% Proteose Peptone #3 (PP3)
(Difco Laboratories, Detroit, MI) liquid media with a primary

35   variant subclone in a 500 ml tribaffled flask with a Delong neck,
covered with a Kaput. Inoculum for each seed culture was derived
from oil-overlay agar slant cultures or plate cultures. After
inoculation, these flasks were incubated for 16 hrs at 28°C on a
rotary shaker at 150 rpm. These seed cultures were then used as

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uniform inoculum sources for a given fermentation of each strain. Additionally, overlaying the post-log seed culture with sterile mineral oil, adding a sterile magnetic stir bar for future resuspension and storing the culture in the dark, at room

5   temperature provided long-term preservation of inoculum in a toxin-competent state. The production broths were inoculated by adding 1% of the actively growing seed culture to fresh 2% PP3 media (e.g., 1.75 ml per 175 ml fresh media). Production of broths occurred in either 500 ml tribaffled flasks (see above), or 2800 ml

10   baffled, convex bottom flasks (500 ml volume) covered by a silicon foam closure. Production flasks were incubated for 24-48 hrs under the above mentioned conditions. Following incubation, the broths were dispensed into sterile 1 L polyethylene bottles, spun at 2600 x g for 1 hr at 10°C and decanted from the cell and debris pellet.

15   The liquid broth was then vacuum filtered through Whatman GF/D (2.7 μM retention) and GF/B (1.0 μM retention) glass filters to remove debris. Further broth clarification was achieved with a tangential flow microfiltration device (Pall Filtron, Northborough, MA) using a 0.5 μM open-channel filter. When necessary, additional

20   clarification could be obtained by chilling the broth (to 4°C) and centrifuging for several hours at 2600 x g. Following these procedures, the broth was filter sterilized using a 0.2 μM nitrocellulose membrane filter. Sterile broths were then used directly for biological assay, biochemical analysis or concentrated

25   (up to 15-fold) using a 10,000 MW cut-off, M12 ultra-filtration device (Amicon, Beverly MA) or centrifugal concentrators (Millipore, Bedford, MA and Pall Filtron, Northborough, MA) with a 10,000 MW pore size. In the case of centrifugal concentrators, the broth was spun at 2000 x g for approximately 2 hr. The 10,000 MW

30   permeate was added to the corresponding retentate to achieve the desired concentration of components greater than 10,000 MW. Heat inactivation of processed broth samples was acheived by heating the samples at 100°C in a sand-filled heat block for 10 minutes.
       The broth(s) and toxin complex(es) from different *Photorhabdus*

35   strains are useful for reducing populations of insects and were used in a method of inhibiting an insect population which comprises applying to a locus of the insect an effective insect inactivating amount of the active described. A demonstration of the breadth of insecticidal activity observed from broths of a selected group of

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Photorhabdus strains fermented as described above is shown in Table
20. It is possible that additional insecticidal activities could
be detected with these strains through increased concentration of
the broth or by employing different fermentation methods.

5   Consistent with the activity being associated with a protein, the
insecticidal activity of all strains tested was heat labile (see
above).

Culture broth(s) from diverse *Photorhabdus* strains show
differential insecticidal activity (mortality and/or growth

10   inhibition, reduced adult emergence) against a number of insects.
More specifically, the activity is seen against corn rootworm
larvae and boll weevil larvae which are members of the insect order
Coleoptera. Other members of the *Coleoptera* include wireworms,
pollen beetles, flea beetles, seed beetles and Colorado potato

15   beetle. Activity is also observed against aster leafhopper and
corn plant hopper, which are members of the order *Homoptera*. Other
members of the *Homoptera* include planthoppers, pear psylla, apple
sucker, scale insects, whiteflies, spittle bugs as well as numerous
host specific aphid species. The broths and purified toxin

20   complex(es) are also active against tobacco budworm, tobacco
hornworm and European corn borer which are members of the order
Lepidoptera. Other typical members of this order are beet
armyworm, cabbage looper, black cutworm, corn earworm, codling
moth, clothes moth, Indian mealmoth, leaf rollers, cabbage worm,

25   cotton bollworm, bagworm, Eastern tent caterpillar, sod webworm and
fall armyworm. Activity is also seen against fruitfly and mosquito
larvae which are members of the order *Diptera*. Other members of
the order *Diptera* are, pea midge, carrot fly, cabbage root fly,
turnip root fly, onion fly, crane fly and house fly and various

30   mosquito species. Activity with broth(s) and toxin complex(es) is
also seen against two-spotted spider mite which is a member of the
order *Acarina* which includes strawberry spider mites, broad mites,
citrus red mite, European red mite, pear rust mite and tomato
russet mite.

35   Activity against corn rootworm larvae was tested as follows.
Photorhabdus culture broth(s) (0-15 fold concentrated, filter
sterilized), 2% Proteose Peptone #3, purified toxin complex(es), 10
mM sodium phosphate buffer , pH 7.0 were applied directly to the
surface (about 1.5 cm^2) of artificial diet (Rose, R. I. and McCabe,

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J. M. (1973). J. Econ. Entomol. 66, (398-400) in 40 μl aliquots.
Toxin complex was diluted in 10 mM sodium phosphate buffer, pH 7.0.
The diet plates were allowed to air-dry in a sterile flow-hood and
the wells were infested with single, neonate *Diabrotica*

5   *undecimpunctata howardi* (Southern corn rootworm, SCR) hatched from
surface sterilized eggs. The plates were sealed, placed in a
humidified growth chamber and maintained at 27°C for the
appropriate period (3-5 days). Mortality and larval weight
determinations were then scored. Generally, 16 insects per

10   treatment were used in all studies. Control mortality was
generally less than 5%.

       Activity against boll weevil (*Anthomonas grandis*) was tested
as follows. Concentrated (1-10 fold) *Photorhabdus* broths, control
medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23

15   mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied in 60
μl aliquots to the surface of 0.35 g of artificial diet (Stoneville
Yellow lepidopteran diet) and allowed to dry. A single, 12-24 hr
boll weevil larva was placed on the diet, and the wells were sealed
and held at 25°C, 50% RH for 5 days. Mortality and larval weights

20   were then assessed. Control mortality ranged between 0-13%.

       Activity against mosquito larvae was tested as follows. The
assay was conducted in a 96-well microtiter plate. Each well
contained 200 μl of aqueous solution (10-fold concentrated
Photorhabdus culture broth(s), control medium (2% Proteose Peptone

25   #3), 10 mM sodium phosphate buffer, toxin complex(es) @ 0.23 mg/ml
or H$_2$0) and approximately 20, 1-day old larvae (*Aedes aegypti*).
There were 6 wells per treatment. The results were read at 3-4
days after infestation. Control mortality was between 0-20%.

       Activity against fruitflies was tested as follows. Purchased

30   *Drosophila melanogaster* medium was prepared using 50% dry medium
and a 50% liquid of either water, control medium (2% Proteose
Peptone #3), 10-fold concentrated *Photorhabdus* culture broth(s),
purified toxin complex(es) [0.23 mg/ml] or 10 mM sodium phosphate
buffer , pH 7.0. This was accomplished by placing 4.0 ml of dry

35   medium in each of 3 rearing vials per treatment and adding 4.0 ml
of the appropriate liquid. Ten late instar *Drosophila melanogaster*
maggots were then added to each 25 ml vial. The vials were held on
a laboratory bench, at room temperature, under fluorescent ceiling
lights. Pupal or adult counts were made after 15 days of exposure.

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Adult emergence as compared to water and control medium (0-16%
reduction).

     Activity against aster leafhopper adults (*Macrosteles
severini) and corn planthopper nymphs (*Peregrinus maidis*) was
5   tested with an ingestion assay designed to allow ingestion of the
active without other external contact. The reservoir for the
active/"food" solution is made by making 2 holes in the center of
the bottom portion of a 35X10 mm Petri dish. A 2 inch Parafilm M®
square is placed across the top of the dish and secured with an "O"
10   ring. A 1 oz. plastic cup is then infested with approximately 7
hoppers and the reservoir is placed on top of the cup, Parafilm
down. The test solution is then added to the reservoir through the
holes. In tests using 10-fold concentrated *Photorhabdus* culture
broth(s), the broth and control medium (2% Proteose Peptone #3)
15   were dialyzed against 10 mM sodium phosphate buffer, pH 7.0 and
sucrose (to 5%) was added to the resulting solution to reduce
control mortality. Purified toxin complex(es) [0.23 mg/ml] or 10
mM sodium phosphate buffer, pH 7.0 was also tested. Mortality is
reported at day 3. The assay was held in an incubator at 28°C, 70%
20   RH with a 16/8 photoperiod. The assays were graded for mortality
at 72 hours. Control mortality was less than 6%.

     Activity against lepidopteran larvae was tested as follows.
Concentrated (10-fold) *Photorhabdus* culture broth(s), control
medium (2% Proteose Peptone #3), purified toxin complex(es) [0.23
25   mg/ml] or 10 mM sodium phosphate buffer, pH 7.0 were applied
directly to the surface (about 1.5 cm^2) of standard artificial
lepidopteran diet (Stoneville Yellow diet) in 40 μl aliquots.   The
diet plates were allowed to air-dry in a sterile flow-hood and each
well was infested with a single, neonate larva. European corn borer
30   (*Ostrinia nubilalis*) and tobacco hornworm (*Manduca sexta)* eggs were
obtained from commercial sources and hatched in-house, whereas
tobacco budworm (*Heliothis virescens*) larvae were supplied
internally. Following infestation with larvae, the diet plates
were sealed, placed in a humidified growth chamber and maintained
35   in the dark at 27°C for the appropriate period. Mortality and
weight determinations were scored at day 5. Generally, 16 insects
per treatment were used in all studies. Control mortality
generally ranged from about 4 to about 12.5% for control medium and
was less than 10% for phosphate buffer.

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Activity against two-spotted spider mite (*Tetranychus urticae*) was determined as follows. Young squash plants were trimmed to a single cotyledon and sprayed to run-off with 10-fold concentrated broth(s), control medium (2% Proteose Peptone #3), purified toxin
5   complex(es), 10 mM sodium phosphate buffer, pH 7.0. After drying, the plants were infested with a mixed population of spider mites and held at lab temperature and humidity for 72 hr. Live mites were then counted to determine levels of control.

Table 20

Observed Insecticidal Spectrum of Broths from
Different _Photorhabdus_ Strains

Photorhabdus Strain	Sensitive* Insect Species
WX-1	3**, 4, 5, 6, 7, 8
WX-2	2, 4
WX-3	1, 4
WX-4	1, 4
WX-5	4
WX-6	4
WX-7	3, 4, 5, 6, 7, 8
WX-8	1, 2, 4
WX-9	1, 2, 4
WX-10	4
WX-11	1, 2, 4
WX-12	2, 4, 5, 6, 7, 8
WX-14	1, 2, 4
WX-15	1, 2, 4
W30	3, 4, 5, 8
NC-1	1, 2, 3, 4, 5, 6, 7, 8, 9
WIR	2, 3, 5, 6, 7, 8
HP88	1, 3, 4, 5, 7, 8
Hb	3, 4, 5, 7, 8
Hm	1, 2, 3, 4, 5, 7, 8
H9	1, 2, 3, 4, 5, 6, 7, 8
W-14	1, 2, 3, 4, 5, 6, 7, 8, 10
ATCC 43948	4
ATCC 43949	4
ATCC 43950	4
ATCC 43951	4
ATCC 43952	4

*   = ≥ 25% mortality and/or growth inhibition vs. control
** = 1; Tobacco budworm, 2; European corn borer, 3;
     Tobacco hornworm, 4; Southern corn rootworm, 5;
     Boll weevil, 6; Mosquito, 7; Fruit Fly, 8;
     Aster Leafhopper, 9; Corn planthopper, 10;
     Two-spotted spider mite.

<u>Example 14</u>
<u>Non W-14 *Photorhabdus* Strains:</u>
<u>Purification, Characterization and Activity Spectrum</u>

5   <u>Purification</u>

        The protocol, as follows, is similar to that developed for the
purification of W-14 and was established based on purifying those
fractions having the most activity against Southern corn root worm
(SCR), as determined in bioassays (see Example 13). Typically, 4-
10   20 L of broth that had been filtered, as described in Example 13,
were received and concentrated using an Amicon spiral ultra
filtration cartridge Type S1Y100 attached to an Amicon M-12
filtration device. The retentate contained native proteins
consisting of molecular sizes greater than 100 kDa, whereas the
15   flow through material contained native proteins less than 100 kDa
in size. The majority of the activity against SCR was contained in
the 100 kDa retentate. The retentate was then continually
diafiltered with 10 mM sodium phosphate (pH = 7.0) until the
filtrate reached an $A_{280} < 0.100$. Unless otherwise stated, all
20   procedures from this point were performed in buffer as defined by
10 mM sodium phosphate (pH 7.0). The retentate was then
concentrated to a final volume of approximately 0.20 L and filtered
using a 0.45 mm Nalgene™ Filterware sterile filtration unit. The
filtered material was loaded at 7.5 ml/min onto a Pharmacia HR16/10
25   column which had been packed with PerSeptive Biosystem Poros® 50 HQ
strong anion exchange matrix equilibrated in buffer using a
PerSeptive Biosystem Sprint® HPLC system. After loading, the
column was washed with buffer until an $A_{280} < 0.100$ was achieved.
Proteins were then eluted from the column at 2.5 ml/min using
30   buffer with 0.4 M NaCl for 20 min for a total volume of 50 ml. The
column was then washed using buffer with 1.0 M NaCl at the same
flow rate for an additional 20 min (final volume = 50 ml).
Proteins eluted with 0.4 M and 1.0 M NaCl were placed in separate
dialysis bags (Spectra/Por® Membrane MWCO: 2,000) and allowed to
35   dialyze overnight at 4° C in 12 L buffer. The majority of the
activity against SCR was contained in the 0.4 M fraction. The 0.4
M fraction was further purified by application of 20 ml to a
Pharmacia XK 26/100 column that had been prepacked with Sepharose
CL4B (Pharmacia) using a flow rate of 0.75 ml/min. Fractions were

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pooled based on A_{280} peak profile and concentrated to a final
volume of 0.75 ml using a Millipore Ultrafree®-15 centrifugal
filter device Biomax-50K NMWL membrane. Protein concentrations
were determined using a Biorad Protein Assay Kit with bovine gamma
5   globulin as a standard.

Characterization

        The native molecular weight of the SCR toxin complex was
determined using a Pharmacia HR 16/50 that had been prepacked with
10 Sepharose CL4B in buffer. The column was then calibrated using
proteins of known molecular size thereby allowing for calculation
of the toxin approximate native molecular size. As shown in Table
21, the molecular size of the toxin complex ranged from 777 kDa
with strain Hb to 1,900 kDa with strain WX-14. The yield of toxin
15 complex also varied, from strain WX-12 producing 0.8 mg/L to strain
Hb, which produced 7.0 mg/L.

        Proteins found in the toxin complex were examined for
individual polypeptide size using SDS-PAGE analysis. Typically, 20
mg protein of the toxin complex from each strain was loaded onto a
20 2-15% polyacrylamide gel (Integrated Separation Systems) and
electrophoresed at 20 mA in Biorad SDS-PAGE buffer. After
completion of electrophoresis, the gels were stained overnight in
Biorad Coomassie blue R-250 (0.2% in methanol: acetic acid: water;
40:10:40 v/v/v). Subsequently, gels were destained in
25 methanol:acetic acid: water; 40:10:40 (v/v/v). The gels were then
rinsed with water for 15 min and scanned using a Molecular Dynamics
Personal Laser Densitometer®. Lanes were quantitated and molecular
sizes were calculated as compared to Biorad high molecular weight
standards, which ranged from 200-45 kDa.
30   Sizes of the individual polypeptides comprising the SCR toxin
complex from each strain are listed in Table 22. The sizes of the
individual polypeptides ranged from 230 kDa with strain WX-1 to a
size of 16 kDa, as seen with strain WX-7. Every strain, with the
exception of strain Hb, had polypeptides comprising the toxin
35 complex that were in the 160-230 kDa range, the 100-160 kDa range,
and the 50-80 kDa range. These data indicate that the toxin
complex may vary in peptide composition and components from strain
to strain, however, in all cases the toxin attributes appears to
consist of a large, oligomeric protein complex.

Table 21

Characterization of a Toxin Complex from

Non W-14 _Photorhabdus_ Strains

5

Strain	Approx. Native Molecular Wt.[a]	Yield Active Fraction (mg/L)[b]
H9	972,000	1.8
Hb	777,000	7.0
Hm	1,400,000	1.1
HP88	813,000	2.5
NC1	1,092,000	3.3
WIR	979,000	1.0
WX-1	973,000	0.8
WX-2	951,000	2.2
WX-7	1,000,000	1.5
WX-12	898,000	0.4
WX-14	1,900,000	1.9
W-14	860,000	7.5

a Native molecular weight determined using a Pharmacia HR
   16/50 column packed with Sepharose CL4B
b Amount of toxin complex recovered from culture broth.

Activity Spectrum

As shown in Table 23, the toxin complexes purified from
10   strains Hm and H9 were tested for activity against a variety of
insects, with the toxin complex from strain W-14 for comparison.
The assays were performed as described in Example 13. The toxin
complex from all three strains exhibited activity against tobacco
bud worm, European corn borer, Southern corn root worm, and aster
15   leafhopper. Furthermore, the toxin complex from strains Hm and W-
14 also exhibited activity against two-spotted spider mite. In
addition, the toxin complex from W-14 exhibited activity against
mosquito larvae. These data indicate that the toxin complex, while
having similarities in activities between certain orders of
20   insects, can also exhibit differential activities against other
orders of insects.

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Table 22

The Approximate Sizes (in kDa) of Peptides in a Purified

Toxin Complex From Non W-14 Photorhabdus

H9	Hb	Hm	HP 88	NC-1	WIR	WX-1	WX-2	WX-7	WX-12	WX-14	W-14
180	150	170	170	180	170	230	200	200	180	210	190
170	140	140	160	170	160	190	170	180	160	180	180
160	139	100	140	140	120	170	150	110	140	160	170
140	130	81	130	110	110	160	120	87	139	120	160
120	120	72	129	44	89	110	110	75	130	110	150
98	100	68	110	16	79	98	82	43	110	100	130
87	98	49	100		74	76	64	33	92	95	120
84	88	46	86		62	58	37	28	87	80	110
79	81	30	81		51	53	30	26	80	69	93
72	75	22	77		40	41		23	73	49	90
68	69	20	73		39	35		22	59	41	77
60	60	19	60		37	31		21	56	33	69
57	57		58		33	28		19	51		65
52	54		45		30	24		18	37		63
46	49		39		28	22		16	33		60
40	44		35		27				32		51
37	39				25				26		46
	37				23						40
	35										39
											29

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Table 23

Observed Insecticidal Spectrum of a Purified Toxin Complex from
Photorhabdus Strains

Photorhabdus Strain	Sensitive* Insect Species
Hm Toxin Complex	1**, 2, 3, 5, 6, 7, 8
H9 Toxin Complex	1, 2, 3, 6, 7, 8
W-14 Toxin Complex	1, 2, 3, 4, 5, 6, 7, 8

* = > 25% mortality or growth inhibition * = > 25% mortality or growth inhibition
** = 1, Tobacco bud worm; 2, European corn borer; 3, Southern corn root worm; 4, Mosquito; 5, Two-spotted spider mite; 6, Aster Leafhopper; 7, Fruit Fly; 8, Boll Weevil

Example 15

Sub-Fractionation of Photorhabdus Protein Toxin Complex

The Photorhabdus protein toxin complex was isolated as
described in Example 14. Next, about 10 mg toxin was applied to a
MonoQ 5/5 column equilibrated with 20 mM Tris-HCl, pH 7.0 at a flow
rate of 1ml/min. The column was washed with 20 mM Tris-HCl, pH 7.0
until the optical density at 280 nm returned to baseline
absorbance. The proteins bound to the column were eluted with a
linear gradient of 0 to 1.0 M NaCl in 20 mM Tris-HCl, pH 7.0 at 1
ml/min for 30 min. One ml fractions were collected and subjected
to Southern corn rootworm (SCR) bioassay (see Example 13). Peaks
of activity were determined by a series of dilutions of each
fraction in SCR bioassays. Two activity peaks against SCR were
observed and were named A (eluted at about 0.2-0.3 M NaCl) and B
(eluted at 0.3-0.4 M NaCl). Activity peaks A and B were pooled
separately and both peaks were further purified using a 3-step
procedure described below.
      Solid $(NH_4)_2SO_4$ was added to the above protein fraction to a
final concentration of 1.7 M. Proteins were then applied to a
phenyl-Superose 5/5 column equilibrated with 1.7 M $(NH_4)_2SO_4$ in 50
mM potassium phosphate buffer, pH 7 at 1 ml/min. Proteins bound to
the column were eluted with a linear gradient of 1.7 M $(NH_4)_2SO_4$,
0% ethylene glycol, 50 mM potassium phosphate, pH 7.0 to 25%
ethylene glycol, 25 mM potassium phosphate, pH 7.0 (no $(NH_4)_2SO_4$)
at 0.5 ml/min. Fractions were dialyzed overnight against 10 mM
sodium phosphate buffer, pH 7.0. Activities in each fraction
against SCR were determined by bioassay.

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The fractions with the highest activity were pooled and
applied to a MonoQ 5/5 column which was equilibrated with 20 mM
Tris-HCl, pH 7.0 at 1 ml/min. The proteins bound to the column
were eluted at 1 ml/min by a linear gradient of 0 to 1M NaCl in 20
5   mM Tris-HCl, pH 7.0.

For the final step of purification, the most active fractions
above (determined by SCR bioassay) were pooled and subjected to a
second phenyl-Superose 5/5/ column. Solid $(NH_4)_2SO_4$ was added to a
final concentration of 1.7 M. The solution was then loaded onto
10   the column equilibrated with 1.7 M $(NH_4)_2SO_4$ in 50 mM potassium
phosphate buffer, pH 7 at 1ml/min. Proteins bound to the column
were eluted with a linear gradient of 1.7 M $(NH_4)_2SO_4$, 50 mM
potassium phosphate, pH 7.0 to 10 mM potassium phosphate, pH 7.0
at 0.5 ml/min. Fractions were dialyzed overnight against 10 mM
15   sodium phosphate buffer, pH 7.0. Activities in each fraction
against SCR were determined by bioassay.

The final purified protein by the above 3-step procedure from
peak A was named toxin A and the final purified protein from peak B
was named toxin B.
20

Characterization and Amino Acid Sequencing of Toxin A and Toxin B

In SDS-PAGE, both toxin A and toxin B contained two major
(> 90% of total Commassie stained protein) peptides: 192 kDa (named
A1 and B1, respectively) and 58 kDa (named A2 and B2,
25   respectively). Both toxin A and toxin B revealed only one major
band in native PAGE, indicating A1 and A2 were subunits of one
protein complex, and B1 and B2 were subunits of one protein
complex. Further, the native molecular weight of both toxin A and
toxin B were determined to be 860 kDa by gel filtration
30   chromatography. The relative molar concentrations of A1 to A2 was
judged to be a 1 to 1 equivalence as determined by densiometric
analysis of SDS-PAGE gels. Similarly, B1 and B2 peptides were
present at the same molar concentration.

Toxin A and toxin B were electrophoresed in 10% SDS-PAGE and
35   transblotted to PVDF membranes. Blots were sent for amino acid
analysis and N-terminal amino acid sequencing at Harvard MicroChem
and Cambridge ProChem, respectively. The N-terminal amino sequence
of B1 was determined to be identical to SEQ ID NO:1, the TcbA$_{ii}$
region of the tcbA gene (SEQ ID NO:12, position 87 to 99). A
40   unique N-terminal sequence was obtained for peptide B2 (SEQ ID
NO:40). The N-terminal amino acid sequence of peptide B2 was
identical to the TcbA$_{iii}$ region of the derived amino acid sequence

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for the *tcbA* gene (SEQ ID NO:12, position 1935 to 1945).
Therefore, the B toxin contained predominantly two peptides, TcbAᵢᵢ
and TcbAᵢᵢᵢ, that were observed to be derived from the same gene
product, TcbA.

5   The N-terminal sequence of A2 (SEQ ID NO:41) was unique in
comparison to the TcbAᵢᵢᵢ peptide and other peptides. The A2
peptide was denoted TcdAᵢᵢᵢ (see Example 17). SEQ ID NO:6 was
determined to be a mixture of amino acid sequences SEQ ID NO:40 and
41.

10   Peptides A1 and A2 were further subjected to internal amino
acid sequencing. For internal amino acid sequencing, 10 μg of
toxin A was electrophoresized in 10% SDS-PAGE and transblotted to
PVDF membrane. After the blot was stained with amido black,
peptides A1 and A2, denoted TcdAᵢᵢ and TcdAᵢᵢᵢ, respectively, were

15   excised from the blot and sent to Harvard MicroChem and Cambridge
ProChem. Peptides were subjected to trypsin digestion followed by
HPLC chromatography to separate individual peptides. N-terminal
amino acid analysis was performed on selected tryptic peptide
fragments. Two internal amino acid sequences of peptide A1

20   (TcdAᵢᵢ-PK71, SEQ ID NO:38 and TcdAᵢᵢ-PK44, SEQ ID NO:39) were
found to have significant homologies with deduced amino acid
sequences of the TcbAᵢᵢ region of the *tcbA* gene (SEQ ID NO:12).
Similarly, the N-terminal sequence (SEQ ID NO:41) and two internal
sequences of peptides A2 (TcdAᵢᵢᵢ-PK57, SEQ ID NO:42 and TcdAᵢᵢᵢ-

25   PK20, SEQ ID NO.43) also showed significant homology with deduced
amino acid sequences of TcbAᵢᵢᵢ region of the *tcbA* gene (SEQ ID
NO:12).

       In summary of above results, the toxin complex has at least
two active protein toxin complexes against SCR; toxin A and toxin

30   B. Toxin A and toxin B are similar in their native and subunits
molecular weight, however, their peptide compositions are
different. Toxin A contained peptides TcdAᵢᵢ and TcdAᵢᵢᵢ as the
major peptides and the toxin B contains TcbAᵢᵢ and TcbAᵢᵢᵢ as the
major peptides.

35

<u>Purification and Characterization of Toxin C, Tca Peptides</u>
       The *Photorhabdus* protein toxin complex was isolated as
described above. Next, about 50 mg toxin was applied to a MonoQ
10/10 column equilibrated with 20 mM Tris-HCl, pH 7.0 at a flow

40   rate of 2 ml/min. The column was washed with 20 mM Tris-HCl, pH7.0

until the optical density at 280 nm returned to baseline level.
The proteins bound to the column were eluted with a linear gradient
of 0 to 1M NaCl in 20 mM Tris-HCl, pH 7.0 at 2 ml/min for 60 min.
2 ml fractions were collected and subjected to Western analysis

5   using pAb TcaB$_{ii}$-syn antibody (see Example 21) as the primary
antibody.   Fractions reacted with pAb TcaB$_{ii}$-syn antibody were
combined and solid $(NH_4)_2SO_4$ was added to a final concentration of
1.7 M.   Proteins were then applied to a phenyl-Superose 10/10
column equilibrated with 1.7 M $(NH_4)_2SO_4$ in 50 mM potassium

10 phosphate buffer, pH 7 at 1ml/min.   Proteins bound to the column
were eluted with a linear gradient of 1.7 M $(NH_4)_2SO_4$, 50 mM
potassium phosphate, pH 7.0 to 10 mM potassium phosphate, pH 7.0
at 1 ml/min for 120 min.   2ml Fractions were collected, dialyzed
overnight against 10 mM sodium phosphate buffer, pH 7.0, and

15 analyzed by Western blots using pAb TcaB$_{ii}$-syn antibody as the
primary antibody.

     Fractions cross-reacted with the antibody were pooled and
applied to a MonoQ 5/5 column which was equilibrated with 20 mM
Tris-HCl, pH 7.0 at 1ml/min.   The proteins bound to the column were

20 eluted at 1ml/min by a linear gradient of 0 to 1M NaCl in 20 mM
Tris-HCl, pH 7.0 for 30 min.

     Fractions above reacted with pAb TcaB$_{ii}$-syn antibody were
pooled and subjected to a phenyl-Superose 5/5/ column.   Solid
$(NH_4)_2SO_4$ added to a final concentration of 1.7 M.   The solution

25 was then applied onto the column equilibrated with 1.7 M $(NH_4)_2SO_4$
in 50 mM potassium phosphate buffer, pH 7 at 1ml/min.   Proteins
bound to the column were then eluted with a linear gradient of 1.7
M $(NH_4)_2SO_4$, 50 mM potassium phosphate, pH 7.0 to 10 mM potassium
phosphate, pH 7.0 at 0.5 ml/min for 60 min.   Fractions were

30 dialyzed overnight against 10 mM sodium phosphate buffer, pH 7.0.
     For the final purification step, fractions reacted with pAb
TcaB$_{ii}$-syn antibody above determined by Western analysis were
combined and applied to a Mono Q 5/5 column equilibrated with 20 mM
Tris-HCl, pH 7.0 at 1ml/min.   The proteins bound to the column were

35 eluted at 1ml/min by a linear gradient of 0 to 1M NaCl in 20 mM
Tris-HCl, pH 7.0 for 30 min.

     The final purified protein fraction contained 6 major peptides
examined by SDS-PAGE: 165 kDa, 90 kDa, 64 kDa, 62 kDa, 58 kDa, and
22 kDa.   The LD50 of the insecticidal activities of this purified

fraction were determined to be 100 ng and 500 ng against SCR and
ECB, respectively.

The above peptides were blotted to PVDF membranes and blots
were sent for amino acids analysis and 5 amino acid long N-terminal
5   sequencing at Harvard MicroChem and Cambridge ProChem,
respectively. The N-terminal amino acid sequence of the 165 kDa
peptide was determined to be identical to peptide TcaC (SEQ ID 2,
position 1 to 5). The N-terminal amino acid sequence of the 90 kDa
peptide was determined to be $TcaA_{ii}$ region of the derived amino
10   acid sequence for the *tcaA* gene (SEQ ID NO 33, position 254 to
258). The N-terminal amino acid sequence of 64 kDa peptide was
determined to be identical to peptide $TcaB_i$ (SEQ ID 3, position 1
to 5). The N-terminal amino acid sequence of the 62 kDa peptide
was determined to be $TcaA_{ii}$ region of the derived amino acid
15   sequence for the *tcaA* gene (SEQ ID NO 33, position 489 to 493).
The N-terminal amino acid sequence of 58 kDa peptide was determined
to be identical to peptide $TcaB_{ii}$ (SEQ ID 5, position 1 to 5). The
N-terminal amino acid sequence of the 22 kDa peptide (SEQ ID NO 62)
was determined to be $TcaA_i$ region, denoted $TcaA_{iv}$, of the derived
20   amino acid sequence for the *tcaA* gene (SEQ ID NO 34, position 98 to
102). It is noted that all *tcaA*, *tcaB*, and *tcaC* genes reside in
the same *tca* operon (Fig. 6A).

Five µg of purified Tca fraction, purified toxin A, and
purified toxin B were analyzed by Western blot using the following
25   antibodies individually as primary antibody: pAb TcaBii-syn
antibody, mAb CF52 antibody, pAb TcdAii-syn antibody, and pAb
Tcd_{iii}-syn antibody (Example 21). With pAb $TcaB_{ii}$-syn antibody
only the purified Tca peptides fraction reacted, but not toxin A or
toxin B. With mAb CF52 antibody, only toxin B reacted but not Tca
30   peptides fraction or toxin A. With either pAb TcdAii-syn antibody
or pAb Tcdiii-syn antibody only toxin A reacted, but not Tca
peptides fraction or toxin B. This indicated that the insecticidal
activity observed in the purified Tca peptides fraction is
independent of toxin A and toxin B. The purified Tca peptide
35   fraction is a third unique protein toxin, denoted toxin C.

<u>Example 16</u>
<u>Cleavage and Activation of TcbA Peptide</u>

5   In the toxin B complex, peptide TcbA$_{ii}$ and TcbA$_{iii}$ originate
   from the single gene product TcbA (Example 15). The processing of
   TcbA peptide to TcbA$_{ii}$ and TcbA$_{iii}$ is presumably by the action of
   Photorhabdus protease(s), and most likely, the metalloproteases
   described in Example 10. In some cases, it was noted that when
   Photorhabdus W-14 broth was processed, TcbA peptide was present in
10 toxin B complex as a major component, in addition to peptides
   TcbA$_{ii}$ and TcbA$_{iii}$. Identical procedures, described for the
   purification of toxin B complex (Example 15), were used to enrich
   peptide TcbA from toxin complex fraction of W-14 broth. The final
   purified material was analyzed in a 4-20% gradient SDS-PAGE and
15 major peptides were quantified by densitometry. It was determined
   that TcbA, TcbA$_{ii}$ and TcbA$_{iii}$ comprised 58%, 36%, and 6%,
   respectively, of total protein. The identities of these peptides
   were confirmed by their respective molecular sizes in SDS-PAGE and
   Western blot analysis using monospecific antibodies. The native
20 molecular weight of this fraction was determined to be 860 kDa.
          The cleavage of TcbA was evaluated by treating the above
   purified material with purified 38 kDa and 58 kDa W-14 *Photorhabdus*
   metalloproteases (Example 10), and trypsin as a control enzyme
   (Sigma, MO). The standard reaction consisted 17.5 μg the above
25 purified fraction, 1.5 unit protease, and 0.1 M Tris buffer, pH 8.0
   in a total volume of 100 μl. For the control reaction, protease
   was omitted. The reaction mixtures were incubated at 37°C for 90
   min. At the end of the reaction, 20 μl was taken and boiled with
   SDS-PAGE sample buffer immediately for electrophoresis analysis in
30 a 4-20% gradient SDS-PAGE. It was determined from SDS-PAGE that in
   both 38 kDa and 58 kDa protease treatments, the amount of peptides
   TcbA$_{ii}$ and TcbA$_{iii}$ increased about 3-fold while the amount of TcbA
   peptide decreased proportionally (Table 24). The relative
   reduction and augmentation of selected peptides was confirmed by
35 Western blot analyses. Furthermore, gel filtration of the cleaved
   material revealed that the native molecular size of the complex
   remained the same. Upon trypsin treatment, peptides TcbA and
   TcbA$_{ii}$ were nonspecifically digested into small peptides. This
   indicated that 38 kDa and 58 kDa *Photorhabdus* proteases can

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specifically process peptide TcbA into peptides TcbA$_{ii}$ and TcbA$_{iii}$.
Protease treated and untreated control of the remaining 80 μl
reaction mixture were serial diluted with 10 mM sodium phosphate
buffer, pH 7.0 and analyzed by SCR bioassay. By comparing activity
5   in several dilution, it was determined that the 38 kDa protease
treatment increased SCR insecticidal activity approximately 3 to 4
fold. The growth inhibition of remaining insects in the protease
treatment was also more severe than control (Table 24).

10   Table 24
Conversion and Activation of Peptide TcbA into Peptides TcbA$_{ii}$ and
TcbA$_{iii}$ by Protease Treatment

	Control	38 kDa protease treatment
TcbA (% of total protein)	58	18
TcbA$_{ii}$ (% of total protein)	36	64
TcbA$_{iii}$ (% of total protein)	6	18
LD50 (μg protein)	2.1	0.52
SCR Weight (mg/insect)*	0.2	0.1

*: an indication of growth inhibition by measuring the average
20 weight of live insect after 5 days on diet in the assay.

Activation and Procession of Toxin B by SCR Gut Proteases
       In yet a second demonstration of proteolytic activation, it
was examined whether W-14 toxins are processed by insects. Toxin B
25 purified from *Photorhabdus* W-14 broth (see Example 15) was
comprised of predominantly intact TcbA peptides as judged by SDS-
PAGE and Western blot analysis using monoclonal antibody. The LD50
of this fraction against SCR was determined to be around 700 ng.
       SCR larva were grown on coleopteran diet until they reached
30 the fourth instar stage (about 100-125 mg total weight each
insect). SCR gut content was collected as follows: the guts were
removed using dissecting scissors and forceps. After removing the
excess fatty material that coats the gut lining, about 40 guts were
homogenized in a microcentrifuge tube containing 100 μl sterile
35 water. The tube was then centrifuged at 14,000 rpm for 10 minutes
and the pellet discarded. The supernatant was stored at a -70°C
freezer until use.
       The processing of toxin B by insect gut was evaluated by
treating the above purified toxin B with the SCR gut content
40 collected. The reaction consisted 40 μg toxin B (1 mg/ml), 50 μl

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SCR gut content, and 0.1M Tris buffer, pH 8.0 in a total volume of
100 μl. For the control reaction, SCR gut content was omitted.
The reaction mixtures were incubated at 37°C for overnight. At the
end of reaction, 10 μl was withdraw and boiled with equal volume 2x

5   SDS-PAGE sample buffer for SDS-PAGE analysis. The remaining 90 μl
reaction mixture was serial diluted with 10 mM sodium phosphate
buffer, pH 7.0 and analyzed by SCR bioassay. SDS-PAGE analysis
indicated in SCR gut content treatment, peptide TcbA was digested
completely into smaller peptides. Analysis of the undenatured

10   toxin fraction showed that the native size, about 860 kDa, remained
the same even though larger peptides were fragmented. In SCR
bioassays, it was found that the LD50 of SCR gut treated toxin B to
be about 70 ng; representing a 10-fold increase. In a separate
experiment, protease K treatment completely eliminated toxin

15   activity.

Example 17
Screening of the Library for a Gene Encoding the TcdA$_{ii}$ Peptide

20   The cloning and characterization of a gene encoding the TcdA$_{ii}$
peptide, described as SEQ ID NO:17 (internal peptide TcdA$_{ii}$-PT111
N-terminal sequence) and SEQ ID NO:18 (internal peptide TcdA$_{ii}$-PT79
N-terminal sequence) was completed. Two pools of degenerate
oligonucleotides, designed to encode the amino acid sequences of

25   SEQ ID NO:17 (Table 25) and SEQ ID NO:18 (Table 26), and the
reverse complements of those sequences, were synthesized as
described in Example 8. The DNA sequence of the oligonucleotides
is given below:

30

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Table 25

Degenerate Oligonucleotide for SEQ ID NO:17

P2-PT111	1	2	3	4	5	6	7	8
Amino Acid	Ala	Phe	Asn	Ile	Asp	Asp	Val	Ser
Codons	5' GCN	TT(T/C)	AA(T/C)	AT(T/C/A)	GA(T/C)	GA(T/C)	GTN 3'	
P2.3.6.CB	5' GC(A/C/G/T)	TT(T/C)	AAT	ATT	GAT	GAT	GT 3'	
P2.3.5	5' GC(A/C/G/T)	TT(T/C)	AA(T/C)	AT(T/C/A)	GA(T/C)	GA(T/C)	GT 3'	
P2.3.5R	5' AC	(G/A)TC	(G/A)TC	(T/G/A)AT	(G/A)TT	(G/A)AA	(A/C/G/T)GC 3'	
P2.3.5RI	5' ACI	TCI	TCI	ATI	TTI	AAI	GC 3'	
P2.3R.CB	5' CAG	(A/G)CT	(A/C)AC	ATC	ATC	AAT	ATT	AAA 3'

Table 26

Degenerate Oligonucleotide for SEQ ID NO:18

P2-PT79	1	2	3	4	5	6	7	8	9	10	11	12	13
Amino Acid	Phe	Ile	Val	Tyr	Thr	Ser	Leu	Gly	Val	Asn	Pro	Asn	Asn
Codons*	5' TTY	ATH	GTN	TAY	ACN	6	6	GGN	GTN	AAY	CCN	AAY	AAY 3'
P2.79.2	5' TTY	ATY	GTK	TAT	ACY	TCI	YTR	GGY	GTK	AAT	CCR	AAT	AAT 3'
P2.79.3	5' TTT	ATT	GTK	TAT	ACY	AGY	YTR	GGY	GTK	AAT	CCR	AAT	AAT 3'
P2.79.R.1	5' ATT	ATT	YGG	ATT	MAC	RCC	YAR	RCT	RGT	ATA	MAC	AAT	AAA 3'
P2.79R.CB	5' ATT	ATT	YGG	ATT	MAC	ACC	CAG	RCT	GGT	ATA	MAC	AAT	AAA 3'

* According to IUPAC-IUB codes for nucleotides, Y = C or T, H = A, C or T,
  N = A, C, G or T, K = G or T, R = A or G, and M = A or C

Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers P2.3.6.CB or P2.3.5, and as reverse primers P2.79.R.1 or P2.79R.CB, in all forward/reverse combinations. In another set of reactions, using Photorhabdus W-14 genomic DNA

5 as template. In another set of reactions, primers P2.79.2 or P2.79.3 were used as forward primers, and P2.3.5R, P2.3.5RI, and P2.3R.CB were used as reverse primers in all forward/reverse combinations. Only in the reactions containing P2.3.6.CB as the forward primers combined with P2.79.R.1 or P2.79R.CB as the reverse

10 primers was a non-artifactual amplified product seen, of estimated size (mobility on agarose gels) of 2500 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment TcdAii-PT111 lies amino-proximal to the peptide fragment TcdAii-PT79.

The 2500 bp PCR products were ligated to the plasmid vector

15 PCR™II (Invitrogen, San Diego, CA) according to the supplier's instructions, and the DNA sequences across the ends of the insert fragments of two isolates (HS24 and HS27) were determined using the supplier's recommended primers and the sequencing methods described previously. The sequence of both isolates was the same. New

20 primers were synthesized based on the determined sequence, and used to prime additional sequencing reactions to obtain a total of 2557 bases of the insert [SEQ ID NO:36]. Translation of the partial peptide encoded by SEQ ID No: 36 yields the 845 amino acid sequence disclosed as SEQ ID NO:37. Protein homology analysis of this

25 portion of the TcdAii peptide fragment reveals substantial amino acid homology ((68% similarity,and 53% identity using the Wisconsin Package Version 8.0, Genetics Computer Group (GCG), Madison, WI) to residues 542 to 1390 of protein TcbA [SEQ ID NO:12] or(60% similarity, and 54% identity using the Wisconsin Package Version

30 9.0, Genetics Computer Group (GCG), Madison, WI to residues 567 to 1389)). It is therefore apparent that the gene represented in part by SEQ ID NO:36 produces a protein of similar, but not identical, amino acid sequence as the TcbA protein, and which likely has similar, but not identical biological activity as the TcbA protein.

35 In yet another instance, a gene encoding the peptides TcdAii-PK44 and the TcdAiii 58 kDa N-terminal peptide, described as SEQ ID NO:39 (internal peptide TcdAii-PK44 sequence), and SEQ ID NO:41(TcdAiii 58 kDa N-terminal peptide sequence) was isolated.

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Two pools of degenerate oligonucleotides, designed to encode the
amino acid sequences described as SEQ ID NO:39 (Table 28) and SEQ
ID NO:41 (Table 27), and the reverse complements of those
sequences, were synthesized as described in Example 8, and their
5   DNA sequences.

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5

Table 27

Degenerate Oligonucleotide for SEQ ID NO:41

Codon #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Amino Acid	Leu	Arg	Ser	Ala	Asn	Thr	Leu	Thr	Asp	Leu	Phe	Leu	Pro	Gln
A2.1	5' YTR	CGY	AGY	GCI	AAT	ACY	YTR	ACY	GAT	YTR	TTT	YTR	CCR	CA 3'
A2.2				GCI	AAT	ACI	YTR	ACI	GAY	YTR	TTY	YTR	CCI	CA 3'
A2.3.R		5' TG	YGG	YAR	AAA	YAR	RTC	RGT	YAR	RGT	RTT	IGC	RCT	RCG 3'
A2.4.R				5' TG	IGG	CAG	AAA	CAG	RTC	IGT	CAG	IGT	ATT	IGC 3'

Table 28

Degenerate Oligonucleotide for SEQ ID NO:39

Amino Acid #	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Codon #	1	2	3	4	5	6	7	8	9
Amino Acid	Gly	Pro	Val	Glu	Ile	Asn	Thr	Ala	Ile
A1.44.1	5' GGY	CCR	GTK	GAA	ATT	AAT	ACC	GCI	AT 3'
A1.44.1R	5' ATI	GCG	GTA	TTA	ATT	TCM	ACY	GGR	CC 3'
A1.44.2	5' GGI	CCI	GTI	GAR	ATY	AAY	ACI	GCI	AT 3'
A1.44.2R	5' ATI	GCI	GTR	TTR	ATY	TCI	ACI	GGI	CC 3'

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Polymerase Chain Reactions (PCR) were performed essentially as described in Example 8, using as forward primers A1.44.1 or A1.44.2, and reverse primers A2.3R or A2.4R, in all forward/reverse combinations, using *Photorhabdus* W-14 genomic DNA as template. In
5   another set of reactions, primers A2.1 or A2.2 were used as forward primers, and A1.44.1R, and A1.44.2R were used as reverse primers in all forward/reverse combinations. Only in the reactions containing A1.44.1 or A1.44.2 as the forward primers combined with A2.3R as the reverse primer was a non-artifactual amplified product seen, of
10   estimated size (mobility on agarose gels) of 1400 base pairs. The order of the primers used to obtain this amplification product indicates that the peptide fragment TcdA$_{ii}$-PK44 lies amino-proximal to the 58 kDa peptide fragment of TcdA$_{iii}$.

The 1400 bp PCR products were ligated to the plasmid vector
15   pCR™II according to the supplier's instructions. The DNA sequences across the ends of the insert fragments of four isolates were determined using primers similar in sequence to the supplier's recommended primers and using sequencing methods described previously. The nucleic acid sequence of all isolates differed as
20   expected in the regions corresponding to the degenerate primer sequences, but the amino acid sequences deduced from these data were the same as the actual amino acid sequences for the peptides determined previously, (SEQ ID NOS:41 and 39).

Screening of the W-14 genomic cosmid library as described in
25   Example 8 with a radiolabeled probe comprised of the DNA prepared above (SEQ ID NO:36) identified five hybridizing cosmid isolates, namely 17D9, 20B10, 21D2, 27B10, and 26D1. These cosmids were distinct from those previously identified with probes corresponding to the genes described as SEQ ID NO:11 or SEQ ID NO:25.
30   Restriction enzyme analysis and DNA blot hybridizations identified three *EcoR I* fragments, of approximate sizes 3.7, 3.7, and 1.1 kbp, that span the region comprising the DNA of SEQ ID NO:36. Screening of the W-14 genomic cosmid library using as probe the radiolabeled 1.4 kbp DNA fragment prepared in this example identified the same
35   five cosmids (17D9, 20B10, 21D2, 27B10, and 26D1). DNA blot hybridization to *EcoR I*-digested cosmid DNAs also showed hybridization to the same subset of *EcoR I* fragments as seen with the 2.5 kbp TcdA$_{ii}$ gene probe, indicating that both fragments are encoded on the genomic DNA.

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DNA sequence determination of the cloned *EcoR I* fragments revealed an uninterrupted reading frame of 7551 base pairs (SEQ ID NO:46), encoding a 282.9 kDa protein of 2516 amino acids (SEQ ID NO:47). Analysis of the amino acid sequence of this protein revealed all expected internal fragments of peptides TcdA$_{ii}$(SEQ ID NOS:17, 18, 37, 38 and 39) and the TcdA$_{iii}$ peptide N-terminus (SEQ ID NO:41) and all TcdA$_{iii}$ internal peptides (SEQ ID NOS:42 and 43). The peptides isolated and identified as TcdA$_{ii}$ and TcdA$_{iii}$ are each products of the open reading frame, denoted *tcdA*, disclosed as SEQ ID NO:46. Further, SEQ ID NO:47 shows, starting at position 89, the sequence disclosed as SEQ ID NO:13, which is the N-terminal sequence of a peptide of size approximately 201 kDa, indicating that the initial protein produced from SEQ ID NO: 46 is processed in a manner similar to that previously disclosed for SEQ ID NO:12. In addition, the protein is further cleaved to generate a product of size 209.2 kDa, encoded by SEQ ID NO:48 and disclosed as SEQ ID NO:49 (TcdA$_{ii}$ peptide), and a product of size 63.6 kDa, encoded by SEQ ID NO:50 and disclosed as SEQ ID NO:51 (TcdA$_{iii}$ peptide). Thus, it is thought that the insecticidal activity identified as toxin A (Example 15) derived from the products of SEQ ID NO:46, as exemplified by the full-length protein of 282.9 kDa disclosed as SEQ ID NO:47, is processed to produce the peptides disclosed as SEQ ID NOS:49 and 51. It is thought that the insecticidal activity identified as toxin B (Example 15) derives from the products of SEQ ID NO:11, as exemplified by the 280.6 kDa protein disclosed as SEQ ID NO:12. This protein is proteolytically processed to yield the 207.6 kDa peptide disclosed as SEQ ID NO:53, which is encoded by SEQ ID NO:52, and the 62.9 kDa peptide having N-terminal sequence disclosed as SEQ ID NO:40, and further disclosed as SEQ ID NO:55, which is encoded by SEQ ID NO:54.

Amino acid sequence comparisons between the proteins disclosed as SEQ ID NO:12 and SEQ ID NO:47 reveal that they have 69% similarity and 54% identity using the Wisconsin Package Version 8.0, Genetics Computer Group (GCG), Madison, WI or 60% similarity and 54% identity using version 9.0 of the program. This high degree of evolutionary relationship is not uniform throughout the entire amino acid sequence of these peptides, but is higher towards the carboxy-terminal end of the proteins, since the peptides disclosed as SEQ ID NO:51 (derived from SEQ ID NO:47) and SEQ ID

NO:55 (derived from SEQ ID NO:12) have 76% similarity and 64%
identity using the Wisconsin Package Version 8.0, Genetics Computer
Group (GCG), Madison, WI or 71% similarity and 64% identity using
version 9.0 of the program.

5

Example 18
Control of European Cornborer-Induced Leaf Damage on Maize Plants
by Spray Application of *Photorhabdus* (Strain W-14) Broth

10

The ability of *Photorhabdus* toxin(s) to reduce plant damage
caused by insect larvae was demonstrated by measuring leaf damage
caused by European corn borer (*Ostrinia nubilalis*) infested onto
maize plants treated with *Photorhabdus* broth. Fermentation broth
15   from *Photorhabdus* strain W-14 was produced and concentrated
approximately 10-fold using ultrafiltration (10,000 MW pore-size)
as described in Example 13. The resulting concentrated broth was
then filter sterilized using 0.2 micron nitrocellulose membrane
filters. A similarly prepared sample of uninoculated 2% proteose
20   peptone #3 was used for control purposes. Maize plants (an inbred
line) were grown from seed to vegetative stage 7 or 8 in pots
containing a soilless mixture in a greenhouse (27°C day; 22°C
night, about 50%RH, 14 hr day-length, watered/fertilized as
needed). The test plants were arranged in a randomized complete
25   block design (3 reps/treatment, 6 plants/treatment) in a greenhouse
with temperature about 22°C day; 18°C night, no artificial light
and with partial shading, about 50%RH and watered/fertilized as
needed. Treatments (uninoculated media and concentrated
Photorhabdus broth) were applied with a syringe sprayer, 2.0 mls
30   applied from directly (about 6 inches) over the whorl and 2.0
additional mls applied in a circular motion from approximately one
foot above the whorl. In addition, one group of plants received no
treatment. After the treatments had dried (approximately 30
minutes), twelve neonate European corn borer larvae (eggs obtained
35   from commercial sources and hatched in-house) were applied directly
to the whorl. After one week, the plants were scored for damage to
the leaves using a modified Guthrie Scale (Koziel, M. G., Beland,
G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L.,
Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis,

K., Maddox, D., McPherson, K., Meghji, M. Z., Merlin, E., Rhodes, R., Warren, G. W., Wright, M. and Evola, S. V. 1993).

Bio/Technology, 11, 194-195.) and the scores were compared statistically [T-test (LSD) $p < 0.05$ and Tukey's Studentized Range

5   (HSD) Test $p < 0.1$]. The results are shown in Table 29. For reference, a score of 1 represents no damage, a score of 2 represents fine "window pane" damage on the unfurled leaf with no pinhole penetration and a score of 5 represents leaf penetration with elongated lesions and/or mid rib feeding evident on more than

10   three leaves (lesions < 1 inch). These data indicate that broth or other protein containing fractions may confer protection against specific insect pests when delivered in a sprayable formulation or when the gene or derivative thereof, encoding the protein or part thereof, is delivered via a transgenic plant or microbe.

15

Table 29

Effect of *Photorhabdus* Culture Broth on

European Corn Borer-Induced Leaf Damage on Maize

20

Treatment	Average Guthrie Score
No Treatment	5.02[a]
Uninoculated medium	5.15[a]
Photorhabdus Broth	2.24[b]

Means with different letters are statistically different
25   ($p < 0.05$ or $p < 0.1$).

Example 19

Genetic Engineering of Genes for Expression in *E. coli*

30   Summary of Constructions

A series of plasmids were constructed to express the *tcbA* gene of *Photorhabdus* W-14 in *Escherichia coli*. A list of the plasmids is shown in Table 30. A brief description of each construction follows as well as a summary of the *E. coli* expression data

35   obtained.

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<u>Table 30</u>

<u>Expression Plasmids for the tcbA Gene</u>

Plasmid	Gene	Vector/Selection	Compartment
pDAB2025	tcbA	pBC/Chl	Intracellular
pDAB2026	tcbA	pAcGP67B/Amp	Baculovirus, secreted
pDAB2027	tcbA	pET27b/Kan	Periplasm
pDAB2028	tcbA	pET15-tcbA	Intracellular

Abbreviations: Kan=kanamycin, Chl=chloramphenicol, Amp=ampicillin

<u>Construction of pDAB2025</u>

In Example 9, a large EcoR I fragment which hybridizes to the TcbA$_{ii}$ probe is described. This fragment was subcloned into pBC (Stratagene, La Jolla CA) to create pDAB2025. Sequence analysis indicates that the fragment is 8816 base pairs. The fragment encodes the tcbA gene with the initiating ATG at position 571 and the terminating TAA at position 8086. The fragment therefore carries 570 base pairs of Photorhabdus DNA upstream of the ATG and 730 base pairs downstream of the TAA.

<u>Construction of Plasmid pDAB2026</u>

The tcbA gene was PCR amplified from plasmid pDAB2025 using the following primers; 5' primer (S1Ac51) 5' TTT AAA CCA TGG GAA ACT CAT TAT CAA GCA CTA TC 3' and 3' primer (S1Ac31) 5' TTT AAA GCG GCC GCT TAA CGG ATG GTA TAA CGA ATA TG 3'. PCR was performed using a TaKaRa LA PCR kit from PanVera (Madison, WI) in the following reaction: 57.5 microliters water, 10 microliters 10X LA buffer, 16 microliters dNTPs (2.5 mM each stock solution), 20 microliters each primer at 10 pmoles/ microliters, 300 ng of the plasmid pDAB2025 containing the W-14 tcbA gene and one microliter of TaKaRa LA Taq polymerase. The cycling conditions were 98°C/20 sec, 68°C/5 min, 72°C/10 min for 30 cycles. A PCR product of the expected about 7526 bp was isolated in a 0.8% agarose gel in TBE (100 mM Tris, 90 mM boric acid, 1 mM EDTA) buffer and purified using a Qiaex II kit from Qiagen (Chatsworth, CA). The purified tcbA gene was digested with Nco I and Not I and ligated into the baculovirus transfer vector pAcGP67B (PharMingen (San Diego, CA)) and transformed into DH5α E. coli. The resulting recombinant is called pDAB2026. The tcbA gene was then cut from pDAB2026 and transferred to pET27b to

create plasmid pDAB2027. A missense mutation in the *tcbA* gene was repaired in pDAB2027.

The repaired *tcbA* gene contains two changes from the sequence shown in Sequence ID NO:11; an A>G at 212 changing an asparagine 71
5   to serine 71 and a G>A at 229 changing an alanine 77 to threonine 77. These changes are both upstream of the proposed TcbA$_{ii}$ N-terminus.

Construction of pDAB2028

10   The *tcbA* coding region of pDAB2027 was transferred to vector pET15b. This was accomplished using shotgun ligations, the DNAs were cut with restriction enzymes *Nco I* and *Xho I*. The resulting recombinant is called pDAB2028.

Expression of TcbA in *E. coli* from Plasmid pDAB2028

15   Expression of *tcbA* in *E. coli* was obtained by modification of the methods previously described by Studier *et al.* (Studier, F.W., Rosenberg, A., Dunn, J., and Dubendorff, J., (1990) Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol.,
20   185: 60-89.). Competent *E. coli* cells strain BL21(DE3) were transformed with plasmid pDAB2028 and plated on LB agar containing 100 μg/mL ampicillin and 40 mM glucose. The transformed cells were plated to a density of several hundred isolated colonies/plate. Following overnight incubation at 37°C the cells were scraped from
25   the plates and suspended in LB broth containing 100 μg/mL ampicillin. Typical culture volumes were from 200-500 mL. At time zero, culture densities (OD600) were from 0.05-0.15 depending on the experiment. Cultures were shaken at one of three temperatures (22°C, 30°C or 37°C) until a density of 0.15-0.5 was obtained at
30   which time they were induced with 1 mM isopropylthio-β-galactoside (IPTG). Cultures were incubated at the designated temperature for 4-5 hours and then were transferred to 4°C until processing (12-72 hours).

Purification and Characterization of TcbA Expressed in *E.coli* from Plasmid pDAB2028

35   *E. coli* cultures expressing TcbA peptides were processed as follows. Cells were harvested by centrifugation at 17,000 x G and the media was decanted and saved in a separate container.

The media was concentrated about 8x using the M12 (Amicon, Beverly MA) filtration system and a 100 kD molecular mass cut-off filter. The concentrated media was loaded onto an anion exchange column and the bound proteins were eluted with 1.0 M NaCl. The 1.0

5   M NaCl elution peak was found to cause mortality against Southern corn rootworm (SCR) larvae Table 30). The 1.0 M NaCl fraction was dialyzed against 10 mM sodium phosphate buffer pH 7.0, concentrated, and subjected to gel filtration on Sepharose CL-4B (Pharmacia, Piscataway, NJ). The region of the CL-4B elution

10   profile corresponding to calculated molecular weight (about 900 kDa) as the native W-14 toxin complex was collected, concentrated and bioassayed against larvae. The collected 900 kDa fraction was found to have insecticidal activity (see Table 31 below), with symptomology similar to that caused by native W-14 toxin complex.

15   This fraction was subjected to Proteinase K and heat treatment, the activity in both cases was either eliminated or reduced, providing evidence that the activity is proteinaceous in nature. In addition, the active fraction tested immunologically positive for the TcbA and TcbA$_{iii}$ peptides in immunoblot analysis when tested

20   with an anti-TcbA$_{iii}$ monoclonal antibody (Table 31).

<div align="center">

Table 31

Results of Immunoblot and SCR Bioassays

</div>

Fraction	SCR Activity		Immunoblot	Native Size
	% Mortality	% Growth Inhibit.	Peptides Detected	[CL-4B Estimated Size]
TcbA Media 1.0 M Ion Exchange	+++	+++	TcbA	
TcbA Media CL-4B	+++	+++	TcbA, TcbA$_{iii}$	about 900 kDa
TcbA Media CL-4B + Proteinase K	++	+++	NT	
TcbA Media CL-4B + heat treatment	-	-	NT	
TcbA Cell Sup CL-4B	-	+++	NT	about 900 kD

25   PK = Proteinase K treatment 2 hours; Heat treatment = 100°C for 10 minutes; ND = None Detected; NT = Not Tested. Scoring system for mortality and growth inhibition as compared to control samples; 5-24%="+", 25-49%="++", 50-100%="+++".

30   The cell pellet was resuspended in 10 mM sodium phosphate buffer, pH=7.0, and lysed by passage through a Bio-Neb™ cell nebulizer (Glas-Col Inc., Terra Haute, IN). The pellets were

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treated with DNase to remove DNA and centrifuged at 17,000 x g to
separate the cell pellet from the cell supernatant. The
supernatant fraction was decanted and filtered through a 0.2 micron
filter to remove large particles and subjected to anion exchange

5   chromatography. Bound proteins were eluted with 1.0 M NaCl,
dialyzed and concentrated using Biomax™ (Millipore Corp, Bedford,
MA) concentrators with a molecular mass cut-off of 50,000 Daltons.
The concentrated fraction was subjected to gel filtration
chromatography using Sepharose CL-4B beaded matrix. Bioassay data

10   for material prepared in this way is shown in Table 30 and is
denoted as "TcbA Cell Sup".

        In yet another method to handle large amounts of material, the
cell pellets were re-suspended in 10 mM sodium phosphate buffer, pH
= 7.0 and thoroughly homogenized by using a Kontes Glass Company

15   (Vineland, NJ) 40 ml tissue grinder. The cellular debris was
pelleted by centrifugation at 25,000 x g and the cell supernatant
was decanted, passed through a 0.2 micron filter and subjected to
anion exchange chromatography using a Pharmacia 10/10 column packed
with Poros HQ 50 beads. The bound proteins were eluted by

20   performing a NaCl gradient of 0.0 to 1.0 M. Fractions containing
the TcbA protein were combined and concentrated using a 50 kDa
concentrator and subjected to gel filtration chromatography using
Pharmacia CL-4B beaded matrix. The fractions containing TcbA
oligomer, molecular mass of approximately 900 kDa, were collected

25   and subjected to anion exchange chromatography using a Pharmacia
Mono Q 10/10 column equilibrated with 20 mM Tris buffer pH = 7.3.
A gradient of 0.0 to 1.0 M NaCl was used to elute recombinant TcbA
protein. Recombinant TcbA eluted from the column at a salt
concentration of approximately 0.3-0.4 M NaCl, the same molarity at

30   which native TcbA oligomer is eluted from the Mono Q 10/10 column.
The recombinant TcbA fraction was found to cause SCR mortality in
bioassay experiments similar to those in Table 31.

        A second set of expression constructions were prepared and tested

35   for expression of the TcbA protein toxin.

Construction of pDAB2030: An Expression Plasmid for the tcbA
Coding Region
        The plasmid pDAB2028 (see herein) contains the tcbA coding

40   region in the commercial vector pET15 (Novagen, Madison, WI),

encodes an ampicillin selection marker. The plasmid pDAB2030 was
created to express the *tcbA* coding region from a plasmid which
encodes a kanamycin selection marker. This was done by cutting
pET27 (Novagen, Madison, WI) a kanamycin selection plasmid, and
5   pDAB2028 with *Xba I* and *Xho I*. This releases the entire multiple
cloning site, including the *tcbA* coding region from plasmid
pDAB2028. The two cut plasmids, were mixed and ligated.
Recombinant plasmids were selected on kanamycin and those
containing the pDAB2028 fragment were identified by restriction
10 analysis. The new recombinant plasmid is called pDAB2030.

Construction of Plasmid pDAB2031: Correction of Mutations in *tcbA*:

        The two mutations in the N-terminus of the *tcbA* coding region
as described in Example 19 (Sequence ID NO:11; A>G at 212 changing
15 an asparagine 71 to serine 71; G>A at 229 changing an alanine 77 to
threonine 77) were corrected as follows: A PCR product was
generated using the primers TH50 (5' ACC GTC TTC TTT ACG ATC AGT G
3')and S1Ac51(5' TTT AAA CCA TGG GAA ACT CAT TAT CAA GCA CTA TC 3')
and pDAB2025 as template to generate a 1778 bp product. This PCR
20 product was cloned into plasmid pCR2.1 (Invitrogen, San Diego, CA)
and a clone was isolated and sequenced. The clone was digested
with *Nco I* and *Pin AI* and a 1670 bp fragment was purified from a 1%
agarose gel. A plasmid containing the mutated *tcbA* coding region
(pDAB2030) was digested with *Nco I* and *Not I* and purified away from
25 the 1670 bp fragment in a 0.8% agarose with Qiaex II (Qiagen,
Chatsworth, CA). The corrected *Nco I/Pin AI* fragment was then
ligated into pDAB2030. The ligated DNA was transformed into DH5α
E. coli. A clone was isolated, sequenced and found to be correct.
This plasmid, containing the corrected *tcbA* coding region, is
30 called pDAB2031.

Construction of pDAB2033 and pDAB2034: Expression Plasmids for
tcbA

        The expression plasmids pDAB2025 and pDAB2027-2031 all rely on
35 the Bacteriophage T7 expression system. An additional vector
system was used for bacterial expression of the *tcbA* gene and its
derivatives. The expression vector Trc99a (Pharmacia Biotech,
Piscataway, NJ) contains a strong trc promoter upstream of a
multiple cloning site with a 5' *Nco I* site which is compatible with
40 the *tcbA* coding region from pDAB2030 and 2031. However, the
plasmid does not have a compatible 3' site. Therefore, the *Hind
III* site of Trc99a was cut and made blunt by treatment with T4 DNA

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polymerase (Boehringer Mannheim, Indianapolis, IN). The vector
plasmid was then cut by *Nco I* followed by treatment with alkaline
phosphatase. The plasmids pDAB2030 and pDAB2031 were each cut with
Xho I (cuts at the 3' end of the *tcbA* coding region) followed by

5   treatment with T4 DNA polymerase to blunt the ends. The plasmids
were then cut with *Nco I*, the DNAs were extracted with phenol,
ethanol precipitated and resuspended in buffer. The Trc99a and
pDAB2030 and pDAB2031 plasmids were mixed separately, ligated and
transformed into DH5α cells and plated on LB media containing

10 ampicillin and 50 mM glucose. Recombinant plasmids were identified
by restriction digestion. The new plasmids are called pDAB2033
(contains the *tcbA* coding sequence with the two mutations in $tcbA_i$)
and pDAB2034 (contains the corrected version of *tcbA* from
pDAB2031).

15

Construction of Plasmid pDAB2032: An Expression Plasmid for $tcbA_{ii}A_{iii}$

A plasmid encoding the $TcbA_{ii}A_{iii}$ portion of TcbA was created
in a similar way as plasmid pDAB2031. A PCR product was generated

20 using TH42 (5' TAG GTC TCC ATG GCT TTT ATA CAA GGT TAT AGT GAT CTG
3') and TH50 (5' ACC GTC TTC TTT ACG ATC AGT G 3') primers and
plasmid pDAB2025 as template. This yielded a product of 1521 bp
having an initiation codon at the beginning of the coding sequence
of $tcbA_{ii}$. This PCR product was isolated in a 1% agarose gel and

25 purified. The purified product was cloned into pCR2.1 as above and
a correct clone was identified by DNA sequence analysis. This
clone was digested with *Nco I* and *Pin AI*, a 1414 bp fragment was
isolated in a 1% agarose gel and ligated into the *Nco I* and *Pin AI*
sites of plasmid pDAB2030 and transformed into DH5α *E. coli*. This

30 new plasmid, designed to express $TcbA_{ii}A_{iii}$ in *E. coli*, is called
pDAB2032.

Expression of *tcbA* and $tcbA_{ii}A_{iii}$ from Plasmids pDAB2030, pDAB2031 and pDAB2032

35 Expression of *tcbA* in *E. coli* from plasmids pDAB2030, pDAB2031
and pDAB2032 was as described herein, except expression of
$tcbA_{ii}A_{iii}$ was done *in E. coli* strain HMS174(DE3)(Novagen, Madison,
WI).

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Expression of tcbA from Plasmid pDAB2033

The plasmid pDAB2033 was transformed into BL21 cells (Novagen, Madison, WI) and plated on LB containing 100 micrograms/mL ampicillin and 50 mM glucose. The plates were spread such that

5   several hundred well separated colonies were present on each plate following incubation at either 30°C or 37°C overnight. The colonies were scraped from the plates and suspended in LB containing 100 micrograms/mL ampicillin, but no glucose. Typical culture volume was 250 mL in a single 1 L baffle bottom flask. The

10   cultures were induced when the culture reached a density of 0.3-0.6 OD600 nm. Most often this density was achieved immediately after suspension of the cells from the plates and did not require a growth period in liquid media. Two induction methods were used. Method 1: cells were induced with 1 mM IPTG at 37°C. The cultures

15   were shaken at 200 rpm on a platform shaker for 5 hours and harvested. Method 2: The cultures were induced with 25 micromolar IPTG at 30°C and shaken at 200 rpm for 15 hours at either 20°C or 30°C. The cultures were stored at 4°C until used for purification.

20 ## Purification of TcbA from E. coli

Purification, bioassay and immunoblot analysis of TcbA and $TcbA_{ii}A_{iii}$ was as described herein. Results of several representative E. coli expression experiments are shown in Table 32. All materials shown in Table 32 were purified from the media

25   fraction of the cultures. The predicted native molecular weight is approximately 900 kD as described herein. The purity of the samples, the amount of TcbA relative to contaminating proteins, varied with each preparation.

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Table 32

Bioassay Activity and Immunoblot Analysis of TcbA and Derivatives
Produced in *E. coli* and Purified from the Culture Media

Plasmid	Coding Region	E. coli Strain	Southern Corn Rootworm Activity		Peptides Detected by Immunoblot	Micrograms Protein Applied to Diet
			% Growth Inhibit.	% Mortal.		
pDAB2030	tcbA	BL21 (DE3)	-	+++	TcbA + TcbA$_{iii}$	1-8
pDAB2031	tcbA	BL21 (DE3)	-	+++	TcbA + TcbA$_{iii}$	1-10
pDAB2033	tcbA	BL21	-	+++	TcbA + TcbA$_{iii}$	1-2
pDAB2032	tcbA$_{ii}$A$_{iii}$	HMS174 (DE3)	+++	+	TcbA$_{ii}$A$_{iii}$ + TcbA$_{iii}$	13-27

Scoring system for mortality and growth inhibition on Southern Corn
Rootworm as compared to control samples; 5-24%="+", 25-49%="++",
50-100%="+++".

Example 20

Characterization of Toxin Peptides with Matrix-Assisted Laser
Desorption Ionization Time-of-Flight Mass Spectroscopy

Toxins isolated from W-14 broth were purified as described in
Example 15. In some cases, the TcaB protein toxin was pretreated
with proteases (Example 16) that had been isolated from W-14 broth
as previously described (Example 15). Protein molecular mass was
determined using matrix-assisted laser desorption ionization time-
of-flight mass spectroscopy, hereinafter MALDI-TOF, on a VOYAGER
BIOSPECTROMETRY workstation with DELAYED EXTRACTION technology
(PerSeptive Biosystems, Framingham, MA). Typically, the protein of
interest (100-500 pmoles in 5 μl) was mixed with 1 μl of
acetonitrile and dialyzed for 0.5 to 1 h on a Millipore VS filter
having a pore size of 0.025 μM (Millipore Corp. Bedford, MA).
Dialysis was performed by floating the filter on water(shinny side
up) followed by adding protein-acetonitrile mixture as a droplet to
the surface of the filter. After dialysis, the dialyzed protein
removed using a pipette and was then mixed with a matrix consisting
of sinapinic acid and trifluoroacetic acid according to
manufacturers instructions. The protein and matrix were allowed to
co-crystallize on a about 3 cm^2 gold-plated sample plate
(PerSeptive Corp.). Excitation of the crystals and subsequent mass
analysis was performed using the following conditions: laser
setting of 3050; pressure of 4.55e-07; low mass gate of 1500.0;
negative ions off; accelerating voltage of 25,000; grid voltage of

-121-

90.0%; guide wire voltage of 0.010%; linear mode; and a pulse delay
time of 350 ns.

 5   Protein mass analysis data are shown in Table 33. The data
obtained from MALDI-TOF was compared to that hypothesized from gene
sequence information and as previously determined by SDS-PAGE.

Table 33
Molecular Analysis of Peptides by MALDI-TOF, SDS-PAGE and Predicted
Determination Based on Gene Sequence

10

Peptide	Predicted (Gene)	SDS PAGE	MALDI-TOF
TcbA	280,634 Da	240,000 Da	281,040 Da
TcbA$_{i/ii}$	217,710 Da	not resolved	216,812 Da
TcbA$_{ii}$	207,698 Da	201,000 Da	206,473 Da
TcbA$_{iii}$	62,943 Da	58,000 Da	63,520 Da
TcdA$_{ii}$	209,218 Da	188,000 Da	208,186 Da
TcdA$_{iii}$	63,520 Da	56,000 Da	63,544 Da
TcbA$_{ii}$ Protease Generated		201,000 Da	216,614 Da^
			215,123 Da^
			210,391 Da^
			208,680 Da^
TcbA$_{iii}$ Protease Generated		56,000 Da	64,111 Da

^Data normalized TcbA, multiple fragments observed at TcbAi/ii

30   Example 21
Production of Peptide Specific Polyclonal Antibodies

        Nine peptide components of the W-14 toxin complex, namely,
TcaA, TcaA$_{iii}$, TcaB$_{i}$, TcaB$_{ii}$, TcaC, TcbA$_{ii}$, TcbA$_{iii}$, TcdA$_{ii}$, and
35   TcdA$_{iii}$ were selected as targets against which antibodies were
produced. Comprehensive DNA and deduced amino acid sequence data
for these peptides indicated that the sequence homology between
some of these peptides was substantial. If a whole peptide was
used as the immunogen to induce antibody production, the resulting
40   antibodies might bind to multiple peptides in the toxin
preparation. To avoid this problem antibodies were generated that
would bind specifically to a unique region of each peptide of
interest. The unique region (subpeptide) of each target peptide
was selected based on the analyses described below.

45   Each entire peptide sequence was analyzed using MacVector™
Protein Analysis Tool (IBI Sequence Analysis Software,
International Biotechnologies, Inc., P. O. Box 9558, New Haven, CT
06535) to determine its antigenicity index. This program was
designed to locate possible externally-located amino acid

sequences, i.e., regions that might be antigenic sites. This
method combined information from hydrophilicity, surface
probability, and backbone flexibility predictions along with the
secondary structure predictions in order to produce a composite

5   prediction of the surface contour of a protein. The scores for
each of the analyses were normalized to a value between -1.0 and

+1.0 (MacVector™ Manual). The antigenicity index value was
obtained for the entire sequence of the target peptide. From each
peptide, an area covering 19 or more amino acids that showed a high

10   antigenicity index from the original sequence was re-analyzed to
determine the antigenicity index of the subpeptide without the
flanking residues. This re-analysis was necessary because the
antigenicity index of a peptide could be influenced by the flanking
amino acid residues. If the isolated subpeptide sequence did not

15   maintain a high antigenicity index, a new region was chosen and the
analysis was repeated.

        Each selected subpeptide sequence was aligned and compared to
all seven target peptide sequences using MacVector™ alignment
program. If a selected subpeptide sequence showed identity

20   (greater than 20%) to another target peptide, a new 19 or more
amino acid region was isolated and re-analyzed. Unique subpeptide
sequences covering 19 or more amino acid showing high antigenicity
index were selected from all target peptides.

        The sequences of seven subpeptides were sent to Genemed

25   Biotechnology Inc. The last amino acid residue on each subpeptide
was deleted because it showed no apparent effect on the
antigenicity index. A cysteine residue was added to the N-terminal
of each subpeptide sequence, except TcaB$_i$-syn which contains an
internal cysteine residue. The present of a cysteine residue

30   facilitates conjugation of a carrier protein (KLH). The final
peptide products corresponding to the appropriate toxin peptides
and SEQ ID NO.s are shown in Table 34.

Table 34
Amino Acid Sequences for Synthetic Peptides

SEQ ID No.	Pepide	Amino Acid Sequence
63	TcaA$_{ii}$-syn	NH2-(C) L R G N S P T N P D K D G I F A Q V A
64	TcaA$_{iii}$-syn	NH2-(C) Y T P D Q T P S F Y E T A F R S A D G
65	TcaB$_{i}$-syn	NH2-H G Q S Y N D N N Y C N F T L S I N T
66	TcaB$_{iii}$-syn	NH2-(C) V D P K T L Q R Q Q A G G D G T G S S
67	TcaC-syn	NH2-(C) Y K A P Q R Q E D G D S N A V T Y D K
68	TcbA$_{ii}$-syn	NH2-(C) Y N E N P S S E D K K W Y F S S K D D
69	TcbA$_{iii}$-syn	NH2-(C) F D S Y S Q L Y E E N I N A G E Q R A
70	TcdA$_{ii}$-syn	NH2-(C) N P N N S S N K L M F Y P V Y Q Y S G N T
71	TcdA$_{iii}$-syn	NH2-(C) V S Q G S G S A G S G N N N L A F G A G

Each conjugated synthetic peptide was injected into two rabbits according to Genemed accelerated program. The pre- and post-immune sera were available for testing after one month.

The preliminary test of both pre- and post-immune sera from each rabbit was performed by Genemed Biotechnologies Inc. Genemed reported that by using both ELISA and Western blot techniques, they detected the reaction of post-immune sera to the respective synthetic peptides. Subsequently, the sera were tested with the whole target peptides, by Western blot analysis. Two batches of partially purified *Photorhabdus* strain W-14 toxin complex was used as the antigen. The two samples had shown activity against the Southern corn rootworm. Their peptide patterns on an SDS-PAGE gel were slightly different.

Pre-cast SDS-polyacrylamide gels with 4-20% gradient (Integrated Separation Systems, Natick, MA 01760) were used. Between 1 to 8 μg of protein was applied to each gel well. Electrophoresis was performed and the protein was electroblotted onto Hybond-ECL$^{\text{TM}}$ nitrocellulose membrane (Amersham International). The membrane was blocked with 10% milk in TBST (25 mM Tris HCl pH 7.4, 136 mM NaCl, 2.7 mM KCl, 0.1% Tween 20) for one hour at room temperature. Each rabbit serum was diluted in 10% milk/TBST to 1:500. Other dilutions between 1:50 to 1:1000 were also used. The serum was added to the membrane and placed on a platform rocker for at least one hour. The membrane was washed thoroughly with the blocking solution or TBST. A 1:2000 dilution of secondary antibodies (goat anti-mouse IgG conjugated to horse radish peroxidase; BioRad Laboratories) in 10% milk/TBST was applied to the membrane placed on a platform rocker for one hour. The membrane was subsequently washed with excess amount of TBST. The

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detection of the protein was performed by using an ECL (Enhanced
Chemiluminescence) detection kit (Amersham International).

Western blot analyses were performed to identify binding
specificity of each anti-synthetic peptide antibodies. All

5   synthetic polyclonal antibodies showed specificity toward to
processed and, when applicable, unprocessed target peptides from
protein fractions derived from *Photorhabdus* culture broth. Various
antibodies were shown to recognize either unprocessed or processed
recombinant proteins derived from heterologous expression systems

10 such as bacteria or insect cells, using baculovirus expression
constructs. In one case, the anti-TcbA$_{iii}$-syn antibody showed some
cross-reactivity to anti-TcdA$_{iii}$ peptide. In a second case, the
anti-TcaC-syn antibody, recognized an unidentified 190 kDa peptide
in W-14 toxin complex fractions.

15

Example 22
Characterization of *Photorhabdus* Strains

In order to establish that the collection described herein was

20 comprised of *Photorhabdus* strains, the strains herein were assessed
in terms of recognized microbiological traits that are
characteristic of the bacterial genus *Photorhabdus* and which
differentiate it from other *Enterobacteriaceae* and *Xenorhabdus* spp.
(Farmer, J. J. 1984. Bergey's Manual of Systemic Bacteriology, Vol

25 1. pp. 510-511. (ed. Kreig N. R. and Holt, J. G.). Williams &
Wilkins, Baltimore.; Akhurst and Boemare, 1988, J. Gen. Microbiol.
134, 1835-1845; Forst and Nealson, 1996. Microbiol. Rev. 60, 21-
43). These characteristic traits are as follows: Gram stain
negative rods, organism size of 0.3-2 μm in width and 2-10 μm in

30 length [with occasional filaments (15-50 μm) and spheroplasts],
yellow to orange/red colony pigmentation on nutrient agar, presence
of crystalline inclusion bodies, presence of catalase, inability to
reduce nitrate, presence of bioluminescence, ability to take up dye
from growth media, positive for protease production, growth at

35 temperatures below 37°C, survival under anaerobic conditions and
positively motile. (Table 33). Test methods were checked using
reference *Escherichia coli*, *Xenorhabdus* and *Photorhabdus* strains.
The overall results are consistent with all strains being part of
the family *Enterobacteriaceae* and the genus *Photorhabdus*. Note

40 that DEP1, DEP2, and DEP3 refer to *Photorhabdus* strains obtained

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from the American Type Culture Collection, 12301 Parklawn Drive,
Rockville, MD 20852 USA (#29304, 29999 and 51583, respectively).

   A luminometer was used to establish the bioluminescence
associated with these *Photorhabdus* strains. To measure the
5   presence or absence of relative light emitting units, the broths
from each strain (cells and media) were measured at three time
intervals after inoculation in liquid culture (24, 48, 72 hr) and
compared to background luminosity (uninoculated media). Several
Xenorhabdus strains were tested as negative controls for
10   luminosity. Prior to measuring light emission from the various
broths, cell density was established by measuring light absorbance
(560 nM) in a Gilford Systems (Oberlin, OH) spectrophotometer using
a sipper cell. The resulting light emitting units could then be
normalized to density of cells. Aliquots of the broths were placed
15   into 96-well microtiter plates (100 μl each) and read in a Packard
Lumicount™ luminometer (Packard Instrument Co., Meriden, CT). The
measurement period for each sample was 0.1 to 1.0 second. The
samples were agitated in the luminometer for 10 sec prior to taking
readings. A positive test was determined as being about 5-fold
20   background luminescence (about 1-15 relative light units). In
addition, degree of colony luminosity was confirmed with
photographic film overlays and by eye, after visual adaptation in a
darkroom. The Gram's staining characteristics of each strain were
established with a commercial Gram's stain kit (BBL, Cockeysville,
25   MD) used in conjunction with Gram's stain control slides (Fisher
Scientific, Pittsburgh, PA). Microscopic evaluation was then
performed using a Zeiss microscope (Carl Zeiss, Germany) 100X oil
immersion objective lens (with 10X ocular and 2X body
magnification). Microscopic examination of individual strains for
30   organism size, cellular description and inclusion bodies (the
latter two observations after logarithmic growth) was performed
using wet mount slides (10X ocular, 2X body and 40X objective
magnification) and phase contrast microscopy with a micrometer
(Akhurst, R. J. and Boemare, N. E. 1990. Entomopathogenic Nematodes
35   in Biological Control (ed. Gaugler, R. and Kaya, H.). pp. 75-90.
CRC Press, Boca Raton, USA.; Baghdiguian S., Boyer-Giglio M. H.,
Thaler, J. O., Bonnot G., Boemare N. 1993. Biol. Cell 79, 177-
185.). Colony pigmentation was observed after inoculation on Bacto
nutrient agar, (Difco Laboratories, Detroit, MI) prepared as per

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label instructions. Incubation occurred at 28°C and descriptions were produced after 5 days. To test for the presence of the enzyme catalase, a colony of the test organism was removed on a small plug from a nutrient agar plate and placed into the bottom of a glass

5   test tube. One ml of a household hydrogen peroxide solution was gently added down the side of the tube. A positive reaction was recorded when bubbles of gas (presumptive oxygen) appeared immediately or within 5 seconds. Controls of uninoculated nutrient agar and hydrogen peroxide solution were also examined. To test

10 for nitrate reduction, each culture was inoculated into 10 ml of Bacto Nitrate Broth (Difco Laboratories, Detroit, MI). After 24 hours incubation with gentle agitation at 28°C, nitrite production was tested by the addition of two drops of sulfanilic acid reagent and two drops of alpha-naphthylamine reagent (see Difco Manual,

15 10th edition, Difco Laboratories, Detroit, MI, 1984). The generation of a distinct pink or red color indicates the formation of nitrite from nitrate whereas the lack of color formation indicates that the strain is nitrate reduction negative. In the latter case, finely powdered zinc was added to further confirm the

20 presence of unreduced nitrate; established by the formation of nitrite and the resultant red color. The ability of each strain to uptake dye from growth media was tested with Bacto MacConkey agar containing the dye neutral red; Bacto Tergitol-7 agar containing the dye bromothymol blue and Bacto EMB Agar containing the dye

25 eosin-Y (formulated agars from Difco Laboratories, Detroit, MI, all prepared according to label instructions). After inoculation on these media, dye uptake was recorded after incubation at 28°C for 5 days. Growth on these latter media is characteristic for members of the family *Enterobacteriaceae*. Motility of each strain was

30 tested using a solution of Bacto Motility Test Medium (Difco Laboratories, Detroit, MI) prepared as per label instructions. A butt-stab inoculation was performed with each strain and motility was judged macroscopically by a diffuse zone of growth spreading from the line of inoculum. The production of protease was tested

35 by observing hydrolysis of gelatin using Bacto gelatin (Difco Laboratories, Detroit, MI) made as per label instructions. Cultures were inoculated and the tubes or plates were incubated at 28°C for 5 days. Gelatin hydrolysis was then checked at room temperature, i.e. less than 22°C. To assess growth at different

temperatures, agar plates [2% proteose peptone #3 with two percent
Bacto-Agar (Difco, Detroit, MI) in deionized water] were streaked
from a common source of inoculum. Plates were incubated at 20, 28
and 37°C for up to three weeks. The incubator temperature levels
5   were checked with an electronic thermocouple and meter to insure
valid temperature settings. Oxygen requirements for *Photorhabdus*
strains were tested in the following manner. A butt-stab
inoculation into fluid thioglycolate broth medium (Difco, Detroit,
MI) was made. The tubes were incubated at room temperature for one
10   week and cultures were then examined for type and extent of growth.
The indicator resazurin demonstrates the presence of medium
oxygenation or the aerobiosis zone (Difco Manual, 10th edition,
Difco Laboratories, Detroit, MI). Growth zone results obtained for
the *Photorhabdus* strains tested were consistent with those of a
15   facultative anaerobic microorganism. In the case of unclear
results, the final agar concentration of fluid thioglycolate broth
medium was raised to 0.75% and the growth characteristics
rechecked.

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<u>Table 35</u>
<u>Taxonomic Traits of *Photorhabdus* Strains</u>

Strain	A*	B	C	D	E	F	G	H	I	J§	K	L	M	N	O	P	Q
P. zealandica	-†	+	+	rd S	+	-	+	+	+	PO	+	+	+	+	+	+	-
P. hepialus	-	+	+	rd S	+	-	+	+	+	Y	+	+	+	+	+	+	-
HB-Arg	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
HB Oswego	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
HB Lewiston	-	+	+	rd S	+	-	+	+	+	T	+	+	+	+	+	+	-
K-122	-	+	+	rd S	+	-	+	+	+	Y	+	+	+	+	+	+	-
HMGD	-	+	+	rd S	+	-	+	+	+	Rd	+	+	+	+	+	+	-
Indicus	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
GD	-	+	+	rd S	+	-	+	+	+	YT	+	+	+	+	+	+	-
PWH-5	-	+	+	rd S	+	-	+	+	+	Y	+	+	+	+	+	+	-
Megidis	-	+	+	rd S	+	-	+	+	+	R	+	+	+	+	+	+	-
HF-85	-	+	+	rd S	+	-	+	+	+	R	+	+	+	+	+	+	-
A. Cows	-	+	+	rd S	+	-	+	+	+	PR	+	+	+	+	+	+	-
MP1	-	+	+	rd S	+	-	+	+	+	T	+	+	+	+	+	+	-
MP2	-	+	+	rd S	+	-	+	+	+	T	+	+	+	+	+	+	-
MP3	-	+	+	rd S	+	-	+	+	+	R	+	+	+	+	+	+	-
MP4	-	+	+	rd S	+	-	+	+	+	Y	+	+	+	+	+	+	-
MP5	-	+	+	rd S	+	-	+	+	+	PR	+	+	+	+	+	+	-
GL98	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
GL101	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
GL138	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
GL155	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
GL217	-	+	+	rd S	+	-	+	+	+	Y	+	+	+	+	+	+	-
GL257	-	+	+	rd S	+	-	+	+	+	O	+	+	+	+	+	+	-
DEP1	-	+	+	rd S	+	-	+	+	+	W	+	+	+	+	+	+	-
DEP2	-	+	+	rd S	+	-	+	+	+	PR	+	+	+	+	+	+	-
DEP3	-	+	+	rd S	+	-	+	+	+	CR	+	+	+	+	+	+	-

5   *: A=Gram's stain, B=Crystaline inclusion bodies,
C=Bioluminescence, D=Cell form, E=Motility, F=Nitrate reduction,
G=Presence of catalase, H=Gelatin hydrolysis, I=Dye uptake,
J=Pigmentation on Nutrient Agar (some color shifts after Day 5),
K=Growth on EMB agar, L=Growth on MacConkey agar, M=Growth on
10   Tergitol-7 agar, N =Facultative anaerobe, O=Growth at 20°C,
P=Growth at 28°C, Q=Growth at 37°C.
†: +=positive for trait, - =negative for trait; rd=rod, S=sized
within Genus descriptors.
§: W = white, CR = cream, Y =yellow, YT=yellow tan, T=tan PO=pale
15   orange, O=orange, PR=pale red, R=red.

The evolutionary diversity of the *Photorhabdus* strains in our
collection was measured by analysis of PCR (Polymerase Chain
Reaction) mediated genomic fingerprinting using genomic DNA from
20   each strain. This technique is based on families of repetitive DNA
sequences present throughout the genome of diverse bacterial
species (reviewed by Versalovic, J., Schneider, M., DE Bruijn,
F. J. and Lupski, J. R. 1994. Methods Mol. Cell. Biol., 5, 25-40).
Three of these, repetitive extragenic palindromic sequence (REP),
25   enterobacterial repetitive intergenic consensus (ERIC) and the BOX

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element are thought to play an important role in the organization
of the bacterial genome. Genomic organization is believed to be
shaped by selection and the differential dispersion of these
elements within the genome of closely related bacterial strains can

5   be used to discriminate these strains (e.g., Louws, F. J.,
Fulbright, D. W., Stephens, C. T. and DE Bruijn, F. J. 1994. Appl.
Environ. Micro. 60, 2286-2295). Rep-PCR utilizes oligonucleotide
primers complementary to these repetitive sequences to amplify the
variably sized DNA fragments lying between them. The resulting

10   products are separated by electrophoresis to establish the DNA
"fingerprint" for each strain.

To isolate genomic DNA from our strains, cell pellets were
resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a
final volume of 10 ml and 12 ml of 5 M NaCl was then added. This

15   mixture was centrifuged 20 min. at 15,000 x g. The resulting
pellet was resuspended in 5.7 ml of TE and 300 μl of 10% SDS and 60
μl 20 mg/ml proteinase K (Gibco BRL Products, Grand Island, NY)
were added. This mixture was incubated at 37°C for 1 hr,
approximately 10 mg of lysozyme was then added and the mixture was

20   incubated for an additional 45 min. One milliliter of 5M NaCl and
800 μl of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl) were then
added and the mixture was incubated 10 min. at 65°C, gently
agitated, then incubated and agitated for an additional 20 min. to
aid in clearing of the cellular material. An equal volume of

25   chloroform/isoamyl alcohol solution (24:1, v/v) was added, mixed
gently then centrifuged. Two extractions were then performed with
an equal volume of phenol/chloroform/isoamyl alcohol (50:49:1).
Genomic DNA was precipitated with 0.6 volume of isopropanol.
Precipitated DNA was removed with a glass rod, washed twice with

30   70% ethanol, dried and dissolved in 2 ml of STE (10 mM Tris-HCl
pH8.0, 10 mM NaCl, 1 mM EDTA). The DNA was then quantitated by
optical density at 260 nm. To perform rep-PCR analysis of
Photorhabdus genomic DNA the following primers were used, REP1R-I;
5'-IIIICGICGICATCIGGC-3' and REP2-I; 5'-ICGICTTATCIGGCCTAC-3'. PCR

35   was performed using the following 25μl reaction: 7.75 μl H$_2$O, 2.5
μl 10X LA buffer (PanVera Corp., Madison, WI), 16 μl dNTP mix (2.5
mM each), 1 μl of each primer at 50 pM/μl, 1 μl DMSO, 1.5 μl
genomic DNA (concentrations ranged from 0.075-0.480 μg/μl) and 0.25
μl TaKaRa EX Taq (PanVera Corp., Madison, WI). The PCR

amplification was performed in a Perkin Elmer DNA Thermal Cycler
(Norwalk, CT) using the following conditions: 95°C/7 min. then 35
cycles of; 94°C/1 min.,44°C/1 min., 65°C/8 min., followed by 15 min.
at 65°C. After cycling, the 25 μl reaction was added to 5 μl of 6X

5   gel loading buffer (0.25% bromophenol blue, 40% w/v sucrose in
H_2O). A 15x20cm 1%-agarose gel was then run in TBE buffer (0.09 M
Tris-borate, 0.002 M EDTA) using 8 μl of each reaction. The gel
was run for approximately 16 hours at 45v. Gels were then stained
in 20 μg/ml ethidium bromide for 1 hour and destained in TBE buffer

10 for approximately 3 hours. Polaroid® photographs of the gels were
then taken under UV illumination.

The presence or absence of bands at specific sizes for each
strain was scored from the photographs and entered as a similarity
matrix in the numerical taxonomy software program, NTSYS-pc (Exeter

15 Software, Setauket, NY). Controls of *E. coli* strain HB101 and
Xanthomonas oryzae pv. *oryzae* assayed under the same conditions
produced PCR fingerprints corresponding to published reports
(Versalovic, J., Koeuth, T. and Lupski, J. R. 1991. Nucleic Acids
Res. 19, 6823-6831; Vera Cruz, C. M., Halda-Alija, L., Louws, F.,

20 Skinner, D. Z., George, M. L., Nelson, R. J., DE Bruijn, F. J.,
Rice, C. and Leach, J. E. 1995. Int. Rice Res. Notes, 20, 23-24.;
Vera Cruz, C. M., Ardales, E. Y., Skinner, D. Z., Talag, J.,
Nelson, R. J., Louws, F. J., Leung, H., Mew, T. W. and Leach, J. E.
1996. Phytopathology 86, 1352-1359). The data from *Photorhabdus*

25 strains were then analyzed with a series of programs within NTSYS-
pc; SIMQUAL (Similarity for Qualitative data) to generate a matrix
of similarity coefficients (using the Jaccard coefficient) and SAHN
(Sequential, Agglomerative, Heirarchical and Nested) clustering
[using the UPGMA (Unweighted Pair-Group Method with Arithmetic

30 Averages) method] which groups related strains and can be expressed
as a phenogram (Fig. 7). The COPH (cophenetic values) and MXCOMP
(matrix comparison) programs were used to generate a cophenetic
value matrix and compare the correlation between this and the
original matrix upon which the clustering was based. A resulting

35 normalized Mantel statistic (r) was generated which is a measure of
the goodness of fit for a cluster analysis (r=0.8-0.9 represents a
very good fit). In our case r=0.924. Therefore, the collection is
comprised of a diverse group of easily distinguishable strains
representative of the *Photorhabdus* genus.

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Example 23
Insecticidal Utility of Toxin(s) Produced
by Various *Photorhabdus* Strains

5

Initial "storage" cultures of the various *Photorhabdus* strains
were produced by inoculating 175 ml of 2% Proteose Peptone #3 (PP3)
(Difco Laboratories, Detroit, MI) liquid medium with a primary
variant colony in a 500 ml tribaffled flask with a Delong neck,

10   covered with a Kaput closure. After inoculation, the flask was
incubated for between 24-72 hrs at 28°C on a rotary shaker at 150
rpm, until stationary phase was reached.   The culture was
transferred to a sterile bottle containing a sterile magnetic stir
bar and the culture was overlayered with sterile mineral oil, to

15   limit exposure to air.   The storage culture was kept in the dark,
at room temperature.   These cultures were then used as inoculum
sources for the fermentation of each strain.
"Seed" flasks or cultures were produced by either inoculating
2 mls of an oil overlayered storage culture or by transferring a

20   primary variant colony into 175 ml sterile medium in a 500 ml
tribaffled flask covered with a Kaput closure. (The use of other
inoculum sources is also possible.) Typically, following 16 hours
incubation at 28°C on a rotary shaker at 150 rpm, the seed culture
was transferred into production flasks. Production flasks were

25   usually inoculated by adding about 1% of the actively growing seed
culture to sterile 2% PP3 medium (e.g. 2.0 ml per 175 ml sterile
medium).   Production of broths occurred in 500 ml tribaffled flasks
covered with a Kaput.   Production flasks were agitated at 28°C on a
rotary shaker at 150 rpm.   Production fermentations were terminated

30   after 24-72 hrs although successful fermentation is not confined to
this time duration. Following appropriate incubation, the broths
were dispensed into sterile 1.0 L polyethylene bottles, spun at
2600xg for 1 hr at 10°C and decanted from the cell and debris
pellet.   Further broth clarification was achieved with a tangential

35   flow microfiltration device (Pall Filtron, Northborough, MA) using
a 0.5 μM open-channel poly-ether sulfone (PES) membrane filter.
The resulting broths were then concentrated (up to 10-fold) using a
10,000 or 100,000 MW cut-off membrane, M12 ultra-filtration device
(Amicon, Beverly MA) or centrifugal concentrators (Millipore,

40   Bedford, MA and Pall Filtron, Northborough, MA) with a 10,000 or

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100,000 MW pore size. In the case of centrifugal concentrators, the broth was spun at 2000xg for approximately 2 hr. The membrane permeate was added to the corresponding retentate to achieve the desired concentration of components greater than the pore size

5   used. Following these procedures, the broth was used for biochemical analysis or filter sterilized using a 0.2 μM cellulose nitrate membrane filter for biological assessment. Heat inactivation of processed broth samples was achieved by heating the samples at 100°C in a sand-filled heat block for 10 minutes.

10   The broth(s) and toxin complex(es) from different *Photorhabdus* strains are useful for reducing populations of insects and were used in a method of inhibiting an insect population which comprises applying to a locus of the insect an effective insect inactivating amount of the active described. A demonstration of the breadth of

15 insecticidal activity observed from broths of a selected group of *Photorhabdus* strains fermented as described above is shown in Table 36. It is possible that improved or additional insecticidal activities could be detected with these strains through increased concentration of the broth or by employing different fermentation

20 methods. Consistent with the activity being associated with a protein, the insecticidal activity of all strains tested was heat labile.

Culture broth(s) from diverse *Photorhabdus* strains show differential insecticidal activity (mortality and/or growth

25 inhibition) against a number of insects. More specifically, the activity is seen against corn rootworm which is a member of the insect order *Coleoptera*. Other members of the *Coleoptera* include boll weevils, wireworms, pollen beetles, flea beetles, seed beetles and Colorado potato beetle. The broths and purified toxin

30 complex(es) are also active against tobacco budworm, tobacco hornworm and European corn borer which are members of the order Lepidoptera. Other typical members of this order are beet armyworm, cabbage looper, black cutworm, corn earworm, codling moth, clothes moth, Indian mealmoth, leaf rollers, cabbage worm,

35 cotton bollworm, bagworm, Eastern tent caterpillar, sod webworm and fall armyworm. Activity is also observed against German cockroach which is a member of the order *Dictyoptera* (or *Blattodea*). Other members of this order are oriental cockroach and American cockroach.

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Activity against corn rootworm larvae was tested as follows.
Photorhabdus culture broth(s) (10 fold concentrated, filter
sterilized), 2% Proteose Peptone #3 (10 fold concentrated),
purified toxin complex(es), 10 mM sodium phosphate buffer, pH 7.0

5   were applied directly to the surface (about 1.5 cm^2) of artificial
diet (Rose, R. I. and McCabe, J. M. 1973. J. Econ. Entomol. 66,
398-400) in 40 μl aliquots. Toxin complex was diluted in 10 mM
sodium phosphate buffer, pH 7.0. The diet plates were allowed to
air-dry in a sterile flow-hood and the wells were infested with

10 single, neonate *Diabrotica undecimpunctata howardi* (Southern corn
rootworm, SCR) hatched from surface sterilized eggs. The plates
were sealed, placed in a humidified growth chamber and maintained
at 27°C for the appropriate period (3-5 days). Mortality and
larval weight determinations were then scored. Generally, 16

15 insects per treatment were used in all studies. Control mortality
was generally less than 5%.

Activity against lepidopteran larvae was tested as follows.
Concentrated (10-fold) *Photorhabdus* culture broth(s), control
medium (2% Proteose Peptone #3), purified toxin complex(es), 10 mM

20 sodium phosphate buffer, pH 7.0 were applied directly to the
surface (about 1.5 cm^2) of standard artificial lepidopteran diet
(Stoneville Yellow diet) in 40 μl aliquots. The diet plates were
allowed to air-dry in a sterile flow-hood and each well was
infested with a single, neonate larva. European corn borer

25 (*Ostrinia nubilalis*) and tobacco hornworm (*Manduca sexta*) eggs were
obtained from commercial sources and hatched in-house, whereas
tobacco budworm (*Heliothis virescens*) larvae were supplied
internally. Following infestation with larvae, the diet plates
were sealed, placed in a humidified growth chamber and maintained

30 in the dark at 27°C for the appropriate period. Mortality and
weight determinations were scored at day 5. Generally, 16 insects
per treatment were used in all studies. Control mortality
generally ranged from about 0 to about 12.5% for control medium and
was less than 10% for phosphate buffer.

35 Activity against cockroach was tested as follows. Concentrated
(10-fold) *Photorhabdus* culture broth(s) and control medium (2%
Proteose Peptone #3) were applied directly to the surface (about
1.5 cm^2) of standard artificial lepidopteran diet (Stoneville
Yellow diet) in 40 μl aliquots. The diet plates were allowed to

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air-dry in a sterile flow-hood and each well was infested with a
single, CO_2 anesthetized first instar German cockroach (*Blatella
germanica*). Following infestation, the diet plates were sealed,
placed in a humidified growth chamber and maintained in the dark at

5   27°C for the appropriate period. Mortality and weight
determinations were scored at day 5. Control mortality less than
10%.

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Table 36

Observed Insecticidal Spectrum of Broths
from Different *Photorhabdus* Strains

Photorhabdus Strain	Sensitive* Insect Species
P. zealandica	1**, 2, 4
P. hepialus	1, 2, 4
HB-Arg	1, 2, 4
HB Oswego	1, 2, 4
HB Lewiston	1, 2, 4
K-122	1, 4
HMGD	1, 4
Indicus	1, 2, 4
GD	2, 4
PWH-5	1, 2, 4
Megidis	1, 2, 4
HF-85	1, 2, 4
A. Cows	1, 4
MP1	1, 2, 4
MP2	1, 2, 4
MP3	4
MP4	1, 4
MP5	4
GL98	1, 4
GL101	1, 4, 5
GL138	1, 2, 4
GL155	1, 4
GL217	1, 2, 4
GL257	1, 4
DEP1	1, 4
DEP2	1, 2, 3, 4
DEP3	4

* = ³ 25% mortality and/or growth inhibition vs. control
** = 1; Tobacco budworm, 2; European corn borer, 3;
      Tobacco hornworm, 4; Southern corn rootworm, 5;
      German cockroach.

Example 24

Southern Analysis of Non-W-14 _Photorhabdus_ Strains

Using W-14 Gene Probes

5   _Photorhabdus_ strais were grown on 2% proteose peptone #3 agar
(Difco Laboratories, Detroit, MI) and insecticidal toxin competence
was maintained by repeated bioassay after passage. A 50 ml shake
culture was produced in 175 ml baffled flasks in 2% proteose
peptone #3 medium, grown at 28° and 150 rpm for approximately 24

10   hours. Fifteen ml of this culture were centrifuged (700 x g, 30
min) and frozen in its medium at -20° until it was thawed (slowly
in ice water) for DNA isolation. The thawed W-14 culture was
centrifuged (900 x g, 15 min 4°), and the floating orange
mucopolysaccharide material was removed. The remaining cell

15   material was centrifuged (25,000 x g, 4°) to pellet the bacterial
cells, and the medium was removed and discarded.
        Total DNA was isolated by an adaptation of the CTAB method
described in section 2.4.1 of Ausubel et al. (1994). The
modifications included a high salt shock, and all volumes were

20   increased ten-fold over the "miniprep" recommended volumes. All
centrifugations were at 4°C unless otherwise specified. The
pelleted bacterial cells were resuspended in TE buffer (10 mM Tris-
HCl, 1 mM EDTA, pH 8) to a final volume of 10 ml, then 12 ml 5 M
NaCl were added; this mixture was centrifuged 20 min at 15,000 x g.

25   The pellet was resuspended in 5.7 ml TE, and 300 μl of 10% SDS and
60 μl of 20 mg/ml proteinase K (in sterile distilled water, Gibco
BRL Products, Grand Island, NY) were added to the suspension. The
mixture was incubated at 37°C for 1 hr; then approximately 10 mg
lysozyme (Worthington Biochemical Corp., Freehold, NJ) were added.

30   After an additional 45 min incubation, 1 ml of 5 M NaCl and 800 μl
of CTAB/NaCl solution (10% w/v CTAB, 0.7 M NaCl) were added. This
preparation was incubated 10 min at 65°C, then gently agitated and
further incubated and agitated for approximately 20 min to assist
clearing of the cellular material. An equal volume of

35   chloroform/isoamyl alcohol solution (24:1, v:v) was added, mixed
very gently, and the phases separated by centrifugation at 12,000 x
g for 15 min. The upper (aqueous) phase was gently removed with a
wide-bore pipette and extracted twice as above with an equal volume
of PCI (phenol/choloroform/ isoamyl alcohol; 50:49:1, v:v:v;

40   equilibrated with 1M Tris-HCl, pH 8.0; Intermountain Scientific
Corporation, Kaysville, UT). The DNA precipitated with 0.6 volume
of isopropanol was gently removed on a glass rod, washed twice with
70% ethanol, dried, and dissolved in 2 ml STE (10 mM Tris-HCl, 10

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mM NaCl, 1 mM EDTA, pH 8). This preparation contained 2.5 mg/ml
DNA, as determined by optical density at 260nm.

Identification of *Bgl II/Hind III* Fragments Hybridizing to *tc*-gene
5 Specific Probes

Approximately 10 µg of genomic DNA was digested to completion
with about 30 units each of *Bgl II* and *Hind III* (NEB) for 180 min,
frozen overnight, then heated at 65°C for five min, and
electrophoresed in a 0.8% agarose gel (Seakem® LE, 1X TEA, 80
10 volts, 90 min). The DNA was stained with ethidium bromide (50
µg/ml) as described earlier, and photographed under ultraviolet
light. The DNA fragments in the agarose gel were subjected to
depurination (5 min in 0.2 M HCl), denaturation (15 min in 0.5 M
NaOH, 1.5 M NaCl), and neutralization (15 min in 0.5 M Tris HCl pH
15 8.0, 1.5 M NaCl), with 3 rinses of distilled water between each
step. The DNA was transferred by Southern blotting from the gel
onto a NYTRAN nylon membrane (Amersham, Arlington Heights, IL)
using a high salt (20X SSC) protocol, as described in section 2.9
of Ausubel et al. (CPMB, op. cit.). The transferred DNA was then
20 UV-crosslinked to the nylon membrane using a Stratagene UV
Stratalinker set on auto crosslink. The membranes were stored dry
at 25°C until use.

Hybidization was performed using the ECL™direct (Amersham,
Arlington Heights, IL) labeling and detection system following
25 protocols provided by the manufacturer. In brief, probes were
prepared by covalently linking the denatured DNA to the enzyme
horseradish peroxidase. Once labeled the probe was used under
hybridization conditions which maintain the enzymatic activity.
Unhybridized probe was removed by two gentle washes 20 minutes each
30 at 42°C in 0.5xSSC, 0.4% SDS, and 6M Urea. This was followed by two
washes 5 minutes each at room temperature in 2xSSC. As directed by
the manufacturer, ECL™ reagents were used to detect the hybridizing
DNA bands. There are several factors which influence the ability
to detect gene relatedness between various *Photorhabdus* strains and
35 strain W-14. First, high stringency conditions have not been
employed in these hybridizations. It is known in the art that
varying the stringency of hybridization and wash conditions will
influence the pattern and intensity of hybridizing bands. Second,
Southern blots' blot to blot variation will influence the mobility
40 of hybridizing bands and molecular weight estamates. Therefore, W-
14 was included as a standard on all Southern blots.

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Gene specific probes derived from the W-14 toxin genes were used in these hybridizations. The following lists the specific coordinates within each gene sequence to which the probe corresponds. A probe specific for $tcaB_i/B_{ii}$: 1174 to 3642 of
5 Sequence ID #25, a probe specific for $tcaC$: 3637 to 6005 of Sequence ID #25, a probe specific for $tcbA$: 2097 to 4964 of Sequence ID #11, and a probe specific for $tcdA$: 1660 to 4191 of sequence ID #46. The following tables summarize Southern Blot analyses of *Photorhabdus* strains. In the event that hybridization
10 of probes occurred, the hybridized fragment(s) were noted as either identical or different from the pattern observed for the W-14 strain.

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Table 37
Southern Analysis of *Photorhabdus* Strains

Strains	tcdA	tcbA	tcaC	tcaB$_{i/ii}$
WX-1	D	D	D	D
WX-2	D	D	-	D
WX-3	D	D	D	D
WX-4	D	D	ND	D
WX-5	D	D	D	D
WX-6	D	D	D	D
WX-7	D	D	ND	D
WX-8	D	D	D	D
WX-9	ND	D	D	D
WX-10	ND	D	D	D
WX-11	ND	D	D	D
WX-12	D	D	D	D
WX-14	D	D	D	D
WX-15	D	D	D	D
HP88	D	-	D	D
Hm	D	-	D	D
Hb	D	-	D	-
H9	D	-	I	D
B2	D	-	D	-
NC-1	D	-	D	D
WIR	D	-	D	D
W30	D	D	D	D
W-14	I	I	I	I

ND = Not determined; - = no detectable hybridization product;
5   I = Identical fragment pattern; D = Different fragment pattern.

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Table 38
Southern Analysis of *Photorhabdus* Strains

Strains	tcdA	tcbA	tcaC	tcaB$_{i/ii}$
K-122	3.3,2.8	D	-	ND
PWH-5	+	D	D	-
Indicus	D	D	3.0	I
Megidis	D	D	D	-
GD	D	D	D	-
HF-85	D	D	D	-
MP 3	D	-	D	-
MP I	D	+	D	-
A. Cows	D	+	D	-
HB-Arg	D	ND	D	-
HMGD	D	D	D	-
HB Lewiston	D	D	D	-
HB Oswego	D	D	D	-
W-14	I	I	I	I

ND = Not determined; - = no detectable hybridization product;
I = Identical fragment pattern; D = Different fragment pattern.
+ = Hybridization fragment pattern not determined.

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Table 39

Southern Analysis of *Photorhabdus* Strains

Strains	tcdA	tcbA	tcaC	$tcaB_i/B_{ii}$
GL98	+	+	D	
GL101	-	+	D	
GL138	-	+	D	
GL155	-	-	-	
GL217	+	-	D	
GL257	+	+	D	
MP4	-	+	-	
MP5	-	-	-	
P hepialus	+	-	D	
P zealandia	+	-	11.0	
DEP1				
DEP2				
DEP3				
W-14	3.8,2.8	2.8	2.8	

ND = Not determined; - = no detectable hybridization product;
5   I = Identical fragment pattern; D = Different fragment pattern.
+ = Hybridization fragment pattern not determined.

From these analyses it is apparent that homologs of W-14 genes
are dispersed throughout these diverse *Photorhabdus* strains, as
10   evidenced by differences in gene fragment sizes between W-14 and
the other strains.

Example 25

N-Terminal Amino Acid Sequences of Toxin Complex Peptides from
15   Different *Photorhabdus* Strains

The relationship of peptides isolated from different
Photorhabdus strains, as described in Example 14, were subjected to

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N-terminal amino acid sequencing. The N-terminal amino acid
sequences of toxin peptides in several strains were compared to W-
14 toxin peptides. In Table 40, a comparison of toxin peptides
compared to date showed that identical or homologous (at least 40%

5   similarity to W14 gene/peptides) toxin peptides were present in all
of the strains. For example, the N-terminal amino acid sequence of
TcaC, SEQ ID NO: 2, was found to be identical to that for 160 kDa
peptide in HP88 but also homologs were present in strains WIR, H9,
Hb, WX-1, and Hm. Some W-14 peptides or homologs have not been

10   observed in other strains; however, not all peptides have been
sequenced for toxin complexes from other strains due to N-terminal
blockage or low abundance. In addition, many other N-terminal
amino acid sequences (SEQ ID NOS: 82 to 88) have been obtained for
toxin complex peptides from other strains that have no similarity

15   to peptides from W-14 and in some case were identical to each
other. For example, an identical amino acid sequence, SEQ ID NO:
82, was obtained for 64 kDa peptide present in both HP88 and Hb
strains and a homologous sequence for a 70 kDa peptide in NC-1
strain (SEQ ID NO: 83).

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Table 40

A Comparison of Amino Terminal Sequence Homology Between Proteins
Isolated From Non-W-14 Strains

W-14 Peptide	W-14 Gene	W-14 SEQ ID	SEQ ID NO:	Strain	Identical	Homology
TcaAii	tcaA	15				---
TcaAiii	tcaA	4				
TcaBi	tcaB	3	76	H9	-	74 kDa
			76	Hm	-	71 kDa
TcaBii	tcaB	5		H9	61 kDa	-
				Hm	61 kDa	-
TcaC	tcaA	2	72	Hb	-	160 kDa
				HP88	160 kDa	-
			73	WIR	-	170 kDa
			74	H9	-	180 kDa
			75	Hm	-	170 kDa
			80	WX-1	-	170 kDa
TcbAii	tcbA	1				
TcbAiii	tcbA	40				
TccA	tccA	8	77	Hb	-	81 kDa
TccB	tccB	7		WX-1	170 kDa	-
				WX-2	180 kDa	-
				WX-14	180 kDa	-
				WIR	170 kDa	-
			78	H9	-	170 kDa
				NC-1	140 kDa	-
			79	Hm	-	190 kDa
TcdAii	tcdA					
TcdAiii	tcdA	41		Hb	57 kDa	-
			81	H9	-	69 kDa
?	?	9		Hb	86 kDa	-
				HP88	86 kDa	-

Homology refers to amino acid sequences that were at least 40%
similarity to W14 gene / peptides. Similar residues were
identified as being a member in one of the following five
groups: (P, A, G, S, T); (Q, N, E, B, D, Z); (H, K, R); (L, I,
V, M); and (F, Y, W).

Example 26

Immunological Analysis of Photorhabdus Strains

        Culture broths of Photorhabdus strains were concentrated 10 to
15 times using Centriprep-10 ultrafiltration device (Amicon, Inc.
Beverly, MA 01915). The concentration of the protein ranges from
0.3 to 3.0 mg per ml. Ten to 20 μg of total protein was loaded in
each well of a precast 4-20% polyacrylamide gel (Integrated
Separation Systems, Natick, MA 01760). Gel electrophoresis was
performed for 1.25 hours using a constant current set at 25 ma per
gel. The gel was electro-blotted on to Hybond-ECL[TM] nitrocellulose
membrane (Amersham Corporation, Arlington Hts, Il 60005) using a
semi-dry electro-blotter (Pharmacia Biotech Inc., Piscataway , NJ

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08854). A constant current was applied at 0.75 ma per cm for 2.5
hours. The membrane was blocked with 10% milk in TBST (25 mM Tris
HCl pH 7.4, 136 mM NaCl, 2.7 mM KCl, 0.1% Tween 20) for one hour at
room temperature. Each primary antibody was diluted in 10%
5   milk/TBST to 1:500. Other dilution between 1:50 to 1:1000 was also
used. The membrane was incubated in primary antibody for at least
one hour. Then it was washed thoroughly with the blocking solution
or TBST. A 1:2000 dilution of secondary antibodies (goat anti-
mouse IgG or goat anti rabbit IgG conjugated to horseradish
10   peroxidase; BioRad Laboratories, Hercules, CA 94547) in 10%
milk/TBST was applied to the membrane which was placed on a
platform rocker for one hour. The membrane was subsequently washed
with excess amount of TBST. The detection of the protein was
performed by using an ECL (Enhanced Chemiluminescence) detection
15   kit (Amersham International).

A panel of peptide specific-antibodies generated against W-14
peptides were used to characterize the protein composition of
broths from nine non-W-14 *Photorhabdus* strains using Western blot
analysis. In addition, one monoclonal antibody (MAb-C5F2) which
20   recognizes TcbA$_{iii}$ protein in W-14-derived toxin complex was used.
The results (Table 39) showed cross recognition of the antibodies
to some of the proteins in these broths. In some cases, the
proteins that were recognized by the antibodies were the same size
as the W-14 target peptides. In other cases, the proteins that
25   were recognized by the antibodies were smaller than the W-14 target
peptides. This data indicate that some of the non-W-14
Photorhabdus strains may produce similar proteins to the W-14
strain. The difference could be due to deletion or protein
processing or degradation process. Some of the strains did not
30   contain protein(s) that could be recognized by some antibodies,
however, it is possible that the concentration is significantly
lower than those observed for W-14 peptides. When compared for
various toxin peptide homologs these results showed peptide
diversity among the *Photorhabdus* strains.

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<u>Table 41</u>

<u>Cross Recognition by Monoclonal Antibodies or Polyclonal Antibodies
Generated Against W-14 Peptides to Protein(s) in Broths of Selected
Non-W-14 *Photorhabdus*</u>

Photo-rhabdus Strain	MAb C5F2	PAb TcdA ii-syn	PAb TcdA iii-syn	PAb TcaC -syn	PAb-TcaB ii-syn	PAb TcbA iii-syn	PAb TcaB i-syn	PAb TcaA ii-syn	PAb TcaA iii-syn
MP1	-	+	+	+	-	+	+	+	+
MP2	+	+	+	+	-	+	+	+	+
MP3	-	+	+	+	-	NT	+	+	-
A. Cows	-	+	+	+	-	NT	+	+	+
Hb-osw	-	-	NT	+	+	NT	+	+	+
H-Arg	-	+	+	+	-	NT	+	+	+
Hb-leu	-	+	+	+	-	NT	+	+	+
Indicus	+	+	+	+	+	NT	+	+	+
HF85	-	+	+	+	-	+	+	+	+
W-14	+	+	+	+	+	+	+	+	+

5   +: Positive reaction; -: Negative reaction; NT: Not Tested

Additional non-W-14 *Photorhabdus* strains were characterized by
Western blot analysis using the culture broth and/or partial

10   purified protein fractions as antigen. The panel of antibodies
include MAb-C5F2, MAb-DE1 (recognizing $TcdA_{ii}$), PAb-DE2 (recognizing
TcaB), PAb-$TcbA_{ii}$-syn, PAb- TcaC-syn, PAb $TcaB_{ii}$-syn, PAb-$TcbA_{iii}$-
syn, PAb-$TcaB_{i}$-syn. These antibodies showed cross-reactivity with
proteins in the broth and in the partial purified fractions of non-

15   W-14 strains.

The data indicate that antibodies could be used to identify
proteins in the broth as well as in the partially purified protein
fractions.

Table 42

Cross Recognition by Monoclonal Antibodies or Polyclonal Antibodies
Generated Against W-14 Peptides to Protein(s) in Broths and/or
Partial Purified Protein Fractions of Selected Non-W14 *Photorhabdus*

Photo-rhabdus Strain	Monoclonal Antibodies		Polyclonal Antibodies					
	Mab C5F2	Mab-DE1	PAb-DE2	PAb TcbA$_{ii}$-syn	PAb TcaC-syn	PAb TcaB$_{ii}$-syn	PAb-TcbA$_{iii}$-syn	PAb-TcaB$_{i}$-syn
WX-1	+	+	+	+	+	+	+	+
WX-2	+	+	+	+	+	+	NT	+
WX-3	+	NT	+	NT	NT	NT	NT	NT
WX-5	+	NT	+	NT	NT	NT	NT	NT
WX-6	+	NT	NT	NT	NT	NT	NT	NT
WX-7	+	+	+	+	+	+	NT	+
WX-8	+	NT	NT	NT	NT	NT	NT	NT
WX-9	+	NT	NT	NT	NT	NT	NT	NT
WX-10	-	NT	NT	NT	NT	NT	NT	NT
WX-12	+	+	+	+	+	+	+	+
WX-14	+	+	+	+	NT	+	NT	+
WX-15	+	NT	NT	NT	NT	NT	NT	NT
W30	+	+	+	NT	NT	NT	NT	NT
Hb	-	NT	+	NT	+	NT	-	+
H9	-	-	+	NT	+	+	NT	NT
Hm	-	NT	+	+	+	+	NT	++
HP88	-	NT	+	-	+	-	-	+
NC-1	+	-	+	+	+	+	NT	+
WIR	-	NT	+	+	+	+	+	+
W-14	+	+	+	+	+	+	+	+

-: Negative reaction; +: Positive reaction; NT: Not tested

Example 27
Bacterial Expression of the *tcdA* Coding Region

Engineering of the *tcdA* Gene for Bacterial Expression

The 5' and 3' ends of the tcdA coding region (SEQ ID NO:46)
were modified to add useful cloning sites for inserting the segment
into heterologous expression vectors. The ends were modified using
unique primers in Polymerase Chain Reactions (PCR), performed
essentially as described in Example 8. Primer sets, as described
below, were used in conjunction with cosmid 21D2.4 as template, to
created products with the appropriately modified ends.

The first primer set was used to modify the 5' end of the gene,
to insert a unique *Nco* I site at the initiator codon using the
forward primer AOF1 (5' GAT CGA TCG ATC CAT GGC CAA CGA GTC TGT AAA
AGA GAT ACC TGA TG TAT TAA AAA GCC AGT GTG 3') and to add unique Bgl
II, Sal I and Not I sites to facilitate insertion of the remainder
of the gene using the reverse primer AOR1 (5' GAT CGA TCG TAC GCG

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GCC GCT CGA TCG ATC GTC GAC CCA TTG ATT TGA GAT CTG GGC GGC GGG TAT
CCA GAT AAT AAA CGG AGT CAC 3').

Another PCR reaction was designed to modify the 3' end of the
gene by adding an additional stop codon and convenient restriction
sites for cloning. The forward primer A0F2 (5' ACT GGC TGC GTG GTC
GAC TGG CGG CGA TTT ACT 3') was used to amplify across a unique Sal
I site in the gene, later used to clone the modified 3' end. The
reverse primer A0R2 (5' CGA TGC ATG CTG CGG CCG CAG GCC TTC CTC GAG
TCA TTA TTT AAT GGT GTA GCG AAT ATG CAA AAT 3') was used to insert a
second stop codon (TGA) and cloning sites *Xho I*, *Stu I* and *Not I*.
Bacterial expression vector pET27b (Novagen, Madison, WI), was
modified to delete the *Bgl II* site at position 446, according to
standard molecular biology techniques.

The 497-bp PCR product from the first amplification reaction
(AOF1+AOR1), to modify the 5' end of the gene, was ligated to the
modified pET27b vector according to the supplier's instructions.
The DNA sequences of the amplified portion of three isolates were
determined using the supplier's recommended primers and the
sequencing methods described previously. The sequence of all
isolates was the same.

One isolate was then used as a cloning vector to insert the
middle portion of the *tcdA* gene on a 6341 bp *Bgl II* to *Sal I*
fragment. The resulting clone was called MC4 and contained all but
the 3' most portion of the *tcdA* coding sequence. Finally, to
complete the full-length coding region, the 832 bp PCR product from
the second PCR amplification (AOF2+AOR2), to modify the 3' end of
the gene, was ligated to isolate MC4 on a *Sal I* to *Not I* fragment,
according to standard molecular biology techniques. The *tcdA* coding
region was sequenced and found to be complete, the resulting plasmid
is called pDAB2035.

Construction of Plasmids pDAB2036, pDAB2037 and pDAB2038 for
Bacterial Expression of *tcdA*

The *tcdA* coding region was cut from plasmid pDAB2035 with
restriction enzymes *Nco I* and *Xho I* and gel purified. The fragment
was ligated into the *Nco I* and *Xho I* sites of the expression vector
pET15 to create plasmid pDAB2036. Additionally, pDAB2035 was cut
with *Nco I* and *Not I* to release the *tcdA* coding region which was
ligated into the *Nco I* and *Not I* sites of the expression vector
pET28b to create plasmid pDAB2037. Finally, plasmid pDAB2035 was
cut with *Nco I* and *Stu I* to release the *tcdA* coding region. This
fragment was ligated into the expression vector Trc99a which was cut
with *Hind III* followed by treatment with T4 DNA polymerase to blunt

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the ends. The vector was then cut with Nco I and ligated with the
Nco I/Stu I cut tcdA fragment. The resulting plasmid is called
pDAB2038.

5   Expression of tcdA from Plasmid pDAB2038
        Plasmid pDAB2038 was transformed into BL21 cells and expressed
as described above for plasmid pDAB2033 in Example 19.

Purification of tcdA from E. coli
10   The expression culture was centrifuged at 10,300 g for 30 min
and the supernatant was collected. It was diluted with two volumes
of H_2O and applied at a flow rate of 7.5 ml/min to a poros 50 HQ
(Perspective Systems, MA) column (1.6 cm x 10 cm) which was pre-
equilibrated with 10 mM sodium phosphate buffer, pH 7.0 (Buffer A).
15 The column was washed with Buffer A until the optical density at 280
nm returned to baseline level. The proteins bound to the column
were then eluted with 1M NaCl in Buffer A.
        The fraction was loaded in 20 ml aliquots onto a gel filtration
column, Sepharose CL-4B (2.6 x 100 cm), which was equilibrated with
20 Buffer A. The protein was eluted in Buffer A at a flow rate of 0.75
mL/min. Fractions with a retention time between 260 minutes and 460
minutes were pooled and applied at 1 mL/min to a Mono Q 5/5 column
which was equilibrated with 20 mM Tris-HCl, pH 7.0 (Buffer B). The
column was washed with Buffer B until the optical density at 280 nm
25 returned to baseline level. The proteins bound to the column were
eluted with a linear gradient of 0 to 1 M NaCl in Buffer B at
1mL/min for 30 min. One milliliter fractions were collected, serial
diluted, and subjected to SCR bioassay. Fractions eluted out
between 0.1 and 0.3 M NaCl were found to have the highest
30 insecticidal activity. Western analysis of the active fractions
using pAb TcdA$_{ii}$-syn antibody and pAb Tcd$_{iii}$-syn antibody indicated
the presence of peptides TcdA$_{ii}$ and TcdA$_{iii}$.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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                    Petell, James
                    Fatig, Raymond
                    Schoonover, Sue
                    ffrench-Constant, Richard
                    Orr, Gregory L
                    Merlo, Donald J
                    Roberts, Jean L
                    Rocheleau, Thomas A

    (ii) TITLE OF INVENTION: Insecticidal Protein Toxins from *Photorhabdus*

  (iii) NUMBER OF SEQUENCES: 88

   (iv) CORRESPONDENCE ADDRESS:
      (A) ADDRESSEE: DowElanco
      (B) STREET: 9330 Zionsville Road
      (C) CITY: Indianapolis
      (D) STATE: IN
      (E) COUNTRY: US
      (F) ZIP: 46268

    (v) COMPUTER READABLE FORM:
      (A) MEDIUM TYPE: Floppy disk
      (B) COMPUTER: IBM PC compatible
      (C) OPERATING SYSTEM: PC-DOS/MS-DOS
      (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

   (vi) CURRENT APPLICATION DATA:
      (A) APPLICATION NUMBER:
      (B) FILING DATE:
      (C) CLASSIFICATION:

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/063,615
      (B) FILING DATE: 18-MAY-1993

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/395,497
      (B) FILING DATE: 28-FEB-1995

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 60/007,255
      (B) FILING DATE: 06-NOV-1995

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/608,423
      (B) FILING DATE: 28-FEB-1996

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/705,484
      (B) FILING DATE: 28-AUG-1996

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/743,699
      (B) FILING DATE: 06-NOV-1996

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```
      (viii) ATTORNEY/AGENT INFORMATION:
             (A) NAME: Borucki, Andrea T.
             (B) REGISTRATION NUMBER: 33651
             (C) REFERENCE/DOCKET NUMBER: 50301E

      (ix) TELECOMMUNICATION INFORMATION:
             (A) TELEPHONE: 317-337-4846
             (B) TELEFAX: 317-337-4847


   (2) INFORMATION FOR SEQ ID NO:1:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 11 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS:
             (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
         (v) FRAGMENT TYPE: N-terminal


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1 (TcbA_{ii} N-terminus):

        Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn
        1               5                   10


   (2) INFORMATION FOR SEQ ID NO:2:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 12 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS:
             (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
         (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2 (TcaC N-terminus):

        Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Trp
        1               5                   10


   (2) INFORMATION FOR SEQ ID NO:3:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 19 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS:
             (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
         (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3 (TcaB_{i} N-terminus):

        Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp Ala
        1               5                   10                  15

        Leu Val Ala
```

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(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 14 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein
    (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4 (TcaA$_{iii}$ N-terminus):

    Ala Ser Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn
    1   5   10

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein
    (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5 (TcaB$_{ii}$ N-terminus):

    Ala Gly Asp Thr Ala Asn Ile Gly Asp
    1   5

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein
    (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

    Leu Gly Gly Ala Ala Thr Leu Leu Asp Leu Leu Leu Pro Gln Ile
    1   5   10   15

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 11 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein
    (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7 (TccB N-terminus):

    Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu
    1   5   10

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(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 9 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS:
       (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: protein
   (v) FRAGMENT TYPE: N-terminal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8 (TccA N-terminus):

Met Asn Leu Ala Ser Pro Leu Ile Ser
1   5

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 16 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS:
       (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: protein
   (v) FRAGMENT TYPE: N-terminal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ile Asn Leu Asp Ile Asn Glu Gln Asn Lys Ile Met Val Val Ser
1   5   10   15

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 20 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS:
       (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: protein
   (v) FRAGMENT TYPE: N-terminal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Ala Lys Asp Val Lys Phe Gly Ser Asp Ala Arg Val Lys Met Leu
1   5   10   15

Arg Gly Val Asn
            20

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 7515 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: DNA (genomic)
  (ix) FEATURE:
       (A) NAME/KEY: CDS
       (B) LOCATION: 1..7515

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11 (*tcbA* gene):

```
        ATG CAA AAC TCA TTA TCA AGC ACT ATC GAT ACT ATT TGT CAG AAA CTG   48
        Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu
  5       1               5                  10                  15

        CAA TTA ACT TGT CCG GCG GAA ATT GCT TTG TAT CCC TTT GAT ACT TTC   96
        Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe
                        20                  25                  30
 10
        CGG GAA AAA ACT CGG GGA ATG GTT AAT TGG GGG GAA GCA AAA CGG ATT  144
        Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile
                    35                  40                  45

 15     TAT GAA ATT GCA CAA GCG GAA CAG GAT AGA AAC CTA CTT CAT GAA AAA  192
        Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys
                50                  55                  60

        CGT ATT TTT GCC TAT GCT AAT CCG CTG CTG AAA AAC GCT GTT CGG TTG  240
 20     Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu
        65                  70                  75                  80

        GGT ACC CGG CAA ATG TTG GGT TTT ATA CAA GGT TAT AGT GAT CTG TTT  288
        Gly Thr Arg Gln Met Leu Gly Phe Ile Gln Gly Tyr Ser Asp Leu Phe
 25                     85                  90                  95

        GGT AAT CGT GCT GAT AAC TAT GCC GCG CCG GGC TCG GTT GCA TCG ATG  336
        Gly Asn Arg Ala Asp Asn Tyr Ala Ala Pro Gly Ser Val Ala Ser Met
                        100                 105                 110
 30
        TTC TCA CCG GCG GCT TAT TTG ACG GAA TTG TAC CGT GAA GCC AAA AAC  384
        Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asn
                        115                 120                 125

 35     TTG CAT GAC AGC AGC TCA ATT TAT TAC CTA GAT AAA CGT CGC CCG GAT  432
        Leu His Asp Ser Ser Ser Ile Tyr Tyr Leu Asp Lys Arg Arg Pro Asp
                130                 135                 140

        TTA GCA AGC TTA ATG CTC AGC CAG AAA AAT ATG GAT GAG GAA ATT TCA  480
 40     Leu Ala Ser Leu Met Leu Ser Gln Lys Asn Met Asp Glu Glu Ile Ser
        145                 150                 155                 160

        ACG CTG GCT CTC TCT AAT GAA TTG TGC CTT GCC GGG ATC GAA ACA AAA  528
        Thr Leu Ala Leu Ser Asn Glu Leu Cys Leu Ala Gly Ile Glu Thr Lys
 45                     165                 170                 175

        ACA GGA AAA TCA CAA GAT GAA GTG ATG GAT ATG TTG TCA ACT TAT CGT  576
        Thr Gly Lys Ser Gln Asp Glu Val Met Asp Met Leu Ser Thr Tyr Arg
                        180                 185                 190
 50
        TTA AGT GGA GAG ACA CCT TAT CAT CAC GCT TAT GAA ACT GTT CGT GAA  624
        Leu Ser Gly Glu Thr Pro Tyr His His Ala Tyr Glu Thr Val Arg Glu
                        195                 200                 205

 55     ATC GTT CAT GAA CGT GAT CCA GGA TTT CGT CAT TTG TCA CAG GCA CCC  672
        Ile Val His Glu Arg Asp Pro Gly Phe Arg His Leu Ser Gln Ala Pro
                210                 215                 220

        ATT GTT GCT GCT AAG CTC GAT CCT GTG ACT TTG TTG GGT ATT AGC TCC  720
 60     Ile Val Ala Ala Lys Leu Asp Pro Val Thr Leu Leu Gly Ile Ser Ser
        225                 230                 235                 240

        CAT ATT TCG CCA GAA CTG TAT AAC TTG CTG ATT GAG GAG ATC CCG GAA  768
        His Ile Ser Pro Glu Leu Tyr Asn Leu Leu Ile Glu Glu Ile Pro Glu
 65                     245                 250                 255

        AAA GAT GAA GCC GCG CTT GAT ACG CTT TAT AAA ACA AAC TTT GGC GAT  816
        Lys Asp Glu Ala Ala Leu Asp Thr Leu Tyr Lys Thr Asn Phe Gly Asp
                        260                 265                 270
 70
        ATT ACT ACT GCT CAG TTA ATG TCC CCA AGT TAT CTG GCC CGG TAT TAT  864
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        Ile Thr Thr Ala Gln Leu Met Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr
                275                 280                 285

 5      GGC GTC TCA CCG GAA GAT ATT GCC TAC GTG ACG ACT TCA TTA TCA CAT  912
        Gly Val Ser Pro Glu Asp Ile Ala Tyr Val Thr Thr Ser Leu Ser His
            290                 295                 300

        GTT GGA TAT AGC AGT GAT ATT CTG GTT ATT CCG TTG GTC GAT GGT GTG  960
10      Val Gly Tyr Ser Ser Asp Ile Leu Val Ile Pro Leu Val Asp Gly Val
        305                 310                 315                 320

        GGT AAG ATG GAA GTA GTT CGT GTT ACC CGA ACA CCA TCG GAT AAT TAT 1008
        Gly Lys Met Glu Val Val Arg Val Thr Arg Thr Pro Ser Asp Asn Tyr
                            325                 330                 335
15

        ACC AGT CAG ACG AAT TAT ATT GAG CTG TAT CCA CAG GGT GGC GAC AAT 1056
        Thr Ser Gln Thr Asn Tyr Ile Glu Leu Tyr Pro Gln Gly Gly Asp Asn
                        340                 345                 350

20      TAT TTG ATC AAA TAC AAT CTA AGC AAT AGT TTT GGT TTG GAT GAT TTT 1104
        Tyr Leu Ile Lys Tyr Asn Leu Ser Asn Ser Phe Gly Leu Asp Asp Phe
                    355                 360                 365

        TAT CTG CAA TAT AAA GAT GGT TCC GCT GAT TGG ACT GAG ATT GCC CAT 1152
25      Tyr Leu Gln Tyr Lys Asp Gly Ser Ala Asp Trp Thr Glu Ile Ala His
            370                 375                 380

        AAT CCC TAT CCT GAT ATG GTC ATA AAT CAA AAG TAT GAA TCA CAG GCG 1200
        Asn Pro Tyr Pro Asp Met Val Ile Asn Gln Lys Tyr Glu Ser Gln Ala
30      385                 390                 395                 400

        ACA ATC AAA CGT AGT GAC TCT GAC AAT ATA CTC AGT ATA GGG TTA CAA 1248
        Thr Ile Lys Arg Ser Asp Ser Asp Asn Ile Leu Ser Ile Gly Leu Gln
                            405                 410                 415
35

        AGA TGG CAT AGC GGT AGT TAT AAT TTT GCC GCC GCC AAT TTT AAA ATT 1296
        Arg Trp His Ser Gly Ser Tyr Asn Phe Ala Ala Ala Asn Phe Lys Ile
                        420                 425                 430

40      GAC CAA TAC TCC CCG AAA GCT TTC CTG CTT AAA ATG AAT AAG GCT ATT 1344
        Asp Gln Tyr Ser Pro Lys Ala Phe Leu Leu Lys Met Asn Lys Ala Ile
                    435                 440                 445

        CGG TTG CTC AAA GCT ACC GGC CTC TCT TTT GCT ACG TTG GAG CGT ATT 1392
45      Arg Leu Leu Lys Ala Thr Gly Leu Ser Phe Ala Thr Leu Glu Arg Ile
            450                 455                 460

        GTT GAT AGT GTT AAT AGC ACC AAA TCC ATC ACG GTT GAG GTA TTA AAC 1440
        Val Asp Ser Val Asn Ser Thr Lys Ser Ile Thr Val Glu Val Leu Asn
50      465                 470                 475                 480

        AAG GTT TAT CGG GTA AAA TTC TAT ATT GAT CGT TAT GGC ATC AGT GAA 1488
        Lys Val Tyr Arg Val Lys Phe Tyr Ile Asp Arg Tyr Gly Ile Ser Glu
                            485                 490                 495
55

        GAG ACA GCC GCT ATT TTG GCT AAT ATT AAT ATC TCT CAG CAA GCT GTT 1536
        Glu Thr Ala Ala Ile Leu Ala Asn Ile Asn Ile Ser Gln Gln Ala Val
                        500                 505                 510

60      GGC AAT CAG CTT AGC CAG TTT GAG CAA CTA TTT AAT CAC CCG CCG CTC 1584
        Gly Asn Gln Leu Ser Gln Phe Glu Gln Leu Phe Asn His Pro Pro Leu
                    515                 520                 525

        AAT GGT ATT CGC TAT GAA ATC AGT GAG GAC AAC TCC AAA CAT CTT CCT 1632
65      Asn Gly Ile Arg Tyr Glu Ile Ser Glu Asp Asn Ser Lys His Leu Pro
            530                 535                 540

        AAT CCT GAT CTG AAC CTT AAA CCA GAC AGT ACC GGT GAT GAT CAA CGC 1680
        Asn Pro Asp Leu Asn Leu Lys Pro Asp Ser Thr Gly Asp Asp Gln Arg
70      545                 550                 555                 560
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AAG GCG GTT TTA AAA CGC GCG TTT CAG GTT AAC GCC AGT GAG TTG TAT 1728
Lys Ala Val Leu Lys Arg Ala Phe Gln Val Asn Ala Ser Glu Leu Tyr
            565   570   575

CAG ATG TTA TTG ATC ACT GAT CGT AAA GAA GAC GGT GTT ATC AAA AAT 1776
Gln Met Leu Leu Ile Thr Asp Arg Lys Glu Asp Gly Val Ile Lys Asn
            580   585   590

AAC TTA GAG AAT TTG TCT GAT CTG TAT TTG GTT AGT TTG CTG GCC CAG 1824
Asn Leu Glu Asn Leu Ser Asp Leu Tyr Leu Val Ser Leu Leu Ala Gln
            595   600   605

ATT CAT AAC CTG ACT ATT GCT GAA TTG AAC ATT TTG TTG GTG ATT TGT 1872
Ile His Asn Leu Thr Ile Ala Glu Leu Asn Ile Leu Leu Val Ile Cys
    610   615   620

GGC TAT GGC GAC ACC AAC ATT TAT CAG ATT ACC GAC GAT AAT TTA GCC 1920
Gly Tyr Gly Asp Thr Asn Ile Tyr Gln Ile Thr Asp Asp Asn Leu Ala
625   630   635   640

AAA ATA GTG GAA ACA TTG TTG TGG ATC ACT CAA TGG TTG AAG ACC CAA 1968
Lys Ile Val Glu Thr Leu Leu Trp Ile Thr Gln Trp Leu Lys Thr Gln
                645   650   655

AAA TGG ACA GTT ACC GAC CTG TTT CTG ATG ACC ACG GCC ACT TAC AGC 2016
Lys Trp Thr Val Thr Asp Leu Phe Leu Met Thr Thr Ala Thr Tyr Ser
            660   665   670

ACC ACT TTA ACG CCA GAA ATT AGC AAT CTG ACG GCT ACG TTG TCT TCA 2064
Thr Thr Leu Thr Pro Glu Ile Ser Asn Leu Thr Ala Thr Leu Ser Ser
            675   680   685

ACT TTG CAT GGC AAA GAG AGT CTG ATT GGG GAA GAT CTG AAA AGA GCA 2112
Thr Leu His Gly Lys Glu Ser Leu Ile Gly Glu Asp Leu Lys Arg Ala
    690   695   700

ATG GCG CCT TGC TTC ACT TCG GCT TTG CAT TTG ACT TCT CAA GAA GTT 2160
Met Ala Pro Cys Phe Thr Ser Ala Leu His Leu Thr Ser Gln Glu Val
705   710   715   720

GCG TAT GAC CTG CTG TTG TGG ATA GAC CAG ATT CAA CCG GCA CAA ATA 2208
Ala Tyr Asp Leu Leu Leu Trp Ile Asp Gln Ile Gln Pro Ala Gln Ile
                725   730   735

ACT GTT GAT GGG TTT TGG GAA GAA GTG CAA ACA ACA CCA ACC AGC TTG 2256
Thr Val Asp Gly Phe Trp Glu Glu Val Gln Thr Thr Pro Thr Ser Leu
                740   745   750

AAG GTG ATT ACC TTT GCT CAG GTG CTG GCA CAA TTG AGC CTG ATC TAT 2304
Lys Val Ile Thr Phe Ala Gln Val Leu Ala Gln Leu Ser Leu Ile Tyr
            755   760   765

CGT CGT ATT GGG TTA AGT GAA ACG GAA CTG TCA CTG ATC GTG ACT CAA 2352
Arg Arg Ile Gly Leu Ser Glu Thr Glu Leu Ser Leu Ile Val Thr Gln
    770   775   780

TCT TCT CTG CTA GTG GCA GGC AAA AGC ATA CTG GAT CAC GGT CTG TTA 2400
Ser Ser Leu Leu Val Ala Gly Lys Ser Ile Leu Asp His Gly Leu Leu
785   790   795   800

ACC CTG ATG GCC TTG GAA GGT TTT CAT ACC TGG GTT AAT GGC TTG GGG 2448
Thr Leu Met Ala Leu Glu Gly Phe His Thr Trp Val Asn Gly Leu Gly
            805   810   815

CAA CAT GCC TCC TTG ATA TTG GCG GCG TTG AAA GAC GGA GCC TTG ACA 2496
Gln His Ala Ser Leu Ile Leu Ala Ala Leu Lys Asp Gly Ala Leu Thr
            820   825   830

GTT ACC GAT GTA GCA CAA GCT ATG AAT AAG GAG GAA TCT CTC CTA CAA 2544
Val Thr Asp Val Ala Gln Ala Met Asn Lys Glu Glu Ser Leu Leu Gln
    835   840   845

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      ATG GCA GCT AAT CAG GTG GAG AAG GAT CTA ACA AAA CTG ACC AGT TGG 2592
      Met Ala Ala Asn Gln Val Glu Lys Asp Leu Thr Lys Leu Thr Ser Trp
          850                 855                 860

      ACA CAG ATT GAC GCT ATT CTG CAA TGG TTA CAG ATG TCT TCG GCC TTG 2640
      Thr Gln Ile Asp Ala Ile Leu Gln Trp Leu Gln Met Ser Ser Ala Leu
      865                 870                 875                 880

10    GCG GTT TCT CCA CTG GAT CTG GCA GGG ATG ATG GCC CTG AAA TAT GGG 2688
      Ala Val Ser Pro Leu Asp Leu Ala Gly Met Met Ala Leu Lys Tyr Gly
                      885                 890                 895

      ATA GAT CAT AAC TAT GCT GCC TGG CAA GCT GCG GCG GCT GCG CTG ATG 2736
15    Ile Asp His Asn Tyr Ala Ala Trp Gln Ala Ala Ala Ala Ala Leu Met
                  900                 905                 910

      GCT GAT CAT GCT AAT CAG GCA CAG AAA AAA CTG GAT GAG ACG TTC AGT 2784
      Ala Asp His Ala Asn Gln Ala Gln Lys Lys Leu Asp Glu Thr Phe Ser
20                915                 920                 925

      AAG GCA TTA TGT AAC TAT TAT ATT AAT GCT GTT GTC GAT AGT GCT GCT 2832
      Lys Ala Leu Cys Asn Tyr Tyr Ile Asn Ala Val Val Asp Ser Ala Ala
          930                 935                 940
25
      GGA GTA CGT GAT CGT AAC GGT TTA TAT ACC TAT TTG CTG ATT GAT AAT 2880
      Gly Val Arg Asp Arg Asn Gly Leu Tyr Thr Tyr Leu Leu Ile Asp Asn
      945                 950                 955                 960

30    CAG GTT TCT GCC GAT GTG ATC ACT TCA CGT ATT GCA GAA GCT ATC GCC 2928
      Gln Val Ser Ala Asp Val Ile Thr Ser Arg Ile Ala Glu Ala Ile Ala
                      965                 970                 975

      GGT ATT CAA CTG TAC GTT AAC CGG GCT TTA AAC CGA GAT GAA GGT CAG 2976
35    Gly Ile Gln Leu Tyr Val Asn Arg Ala Leu Asn Arg Asp Glu Gly Gln
                      980                 985                 990

      CTT GCA TCG GAC GTT AGT ACC CGT CAG TTC TTC ACT GAC TGG GAA CGT 3024
      Leu Ala Ser Asp Val Ser Thr Arg Gln Phe Phe Thr Asp Trp Glu Arg
40                995                 1000                1005

      TAC AAT AAA CGT TAC AGT ACT TGG GCT GGT GTC TCT GAA CTG GTC TAT 3072
      Tyr Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr
                1010                1015                1020
45
      TAT CCA GAA AAC TAT GTT GAT CCC ACT CAG CGC ATT GGG CAA ACC AAA 3120
      Tyr Pro Glu Asn Tyr Val Asp Pro Thr Gln Arg Ile Gly Gln Thr Lys
      1025                1030                1035                1040

50    ATG ATG GAT GCG CTG TTG CAA TCC ATC AAC CAG AGC CAG CTA AAT GCG 3168
      Met Met Asp Ala Leu Leu Gln Ser Ile Asn Gln Ser Gln Leu Asn Ala
                      1045                1050                1055

      GAT ACG GTG GAA GAT GCT TTC AAA ACT TAT TTG ACC AGC TTT GAG CAG 3216
55    Asp Thr Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Ser Phe Glu Gln
                      1060                1065                1070

      GTA GCA AAT CTG AAA GTA ATT AGT GCT TAC CAC GAT AAT GTG AAT GTG 3264
      Val Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Val Asn Val
60                1075                1080                1085

      GAT CAA GGA TTA ACT TAT TTT ATC GGT ATC GAC CAA GCA GCT CCG GGT 3312
      Asp Gln Gly Leu Thr Tyr Phe Ile Gly Ile Asp Gln Ala Ala Pro Gly
          1090                1095                1100
65
      ACG TAT TAC TGG CGT AGT GTT GAT CAC AGC AAA TGT GAA AAT GGC AAG 3360
      Thr Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Cys Glu Asn Gly Lys
      1105                1110                1115                1120

70    TTT GCC GCT AAT GCT TGG GGT GAG TGG AAT AAA ATT ACC TGT GCT GTC 3408
      Phe Ala Ala Asn Ala Trp Gly Glu Trp Asn Lys Ile Thr Cys Ala Val
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                    1125                      1130                    1135

        AAT CCT TGG AAA AAT ATC ATC CGT CCG GTT GTT TAT ATG TCC CGC TTA 3456
        Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu
   5                    1140                    1145                1150

        TAT CTG CTA TGG CTG GAG CAG CAA TCA AAG AAA AGT GAT GAT GGT AAA 3504
        Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys
                        1155                    1160                1165
  10
        ACC ACG ATT TAT CAA TAT AAC TTA AAA CTG GCT CAT ATT CGT TAC GAC 3552
        Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp
                    1170                    1175                1180

  15    GGT AGT TGG AAT ACA CCA TTT ACT TTT GAT GTG ACA GAA AAG GTA AAA 3600
        Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys
        1185                    1190                    1195            1200

        AAT TAC ACG TCG AGT ACT GAT GCT GCT GAA TCT TTA GGG TTG TAT TGT 3648
        Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys
  20                    1205                    1210                1215

        ACT GGT TAT CAA GGG GAA GAC ACT CTA TTA GTT ATG TTC TAT TCG ATG 3696
        Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met
  25                    1220                    1225                1230

        CAG AGT AGT TAT AGC TCC TAT ACC GAT AAT AAT GCG CCG GTC ACT GGG 3744
        Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly
                        1235                    1240                1245
  30
        CTA TAT ATT TTC GCT GAT ATG TCA TCA GAC AAT ATG ACG AAT GCA CAA 3792
        Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln
                    1250                    1255                    1260

  35    GCA ACT AAC TAT TGG AAT AAC AGT TAT CCG CAA TTT GAT ACT GTG ATG 3840
        Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met
        1265                    1270                    1275            1280

        GCA GAT CCG GAT AGC GAC AAT AAA AAA GTC ATA ACC AGA AGA GTT AAT 3888
  40    Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn
                        1285                    1290                1295

        AAC CGT TAT GCG GAG GAT TAT GAA ATT CCT TCC TCT GTG ACA AGT AAC 3936
        Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn
  45                    1300                    1305                1310

        AGT AAT TAT TCT TGG GGT GAT CAC AGT TTA ACC ATG CTT TAT GGT GGT 3984
        Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly
                        1315                    1320                1325
  50
        AGT GTT CCT AAT ATT ACT TTT GAA TCG GCG GCA GAA GAT TTA AGG CTA 4032
        Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu
                    1330                    1335                    1340

  55    TCT ACC AAT ATG GCA TTG AGT ATT ATT CAT AAT GGA TAT GCG GGA ACC 4080
        Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr
        1345                    1350                    1355            1360

        CGC CGT ATA CAA TGT AAT CTT ATG AAA CAA TAC GCT TCA TTA GGT GAT 4128
  60    Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp
                        1365                    1370                1375

        AAA TTT ATA ATT TAT GAT TCA TCA TTT GAT GAT GCA AAC CGT TTT AAT 4176
        Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn
  65                    1380                    1385                1390

        CTG GTG CCA TTG TTT AAA TTC GGA AAA GAC GAG AAC TCA GAT GAT AGT 4224
        Leu Val Pro Leu Phe Lys Phe Gly Lys Asp Glu Asn Ser Asp Asp Ser
                        1395                    1400                1405
  70
        ATT TGT ATA TAT AAT GAA AAC CCT TCC TCT GAA GAT AAG AAG TGG TAT 4272
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              Ile Cys Ile Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr
                  1410                1415                1420

  5           TTT TCT TCG AAA GAT GAC AAT AAA ACA GCG GAT TAT AAT GGT GGA ACT 4320
              Phe Ser Ser Lys Asp Asp Asn Lys Thr Ala Asp Tyr Asn Gly Gly Thr
                  1425            1430                1435                1440

              CAA TGT ATA GAT GCT GGA ACC AGT AAC AAA GAT TTT TAT TAT AAT CTC 4368
              Gln Cys Ile Asp Ala Gly Thr Ser Asn Lys Asp Phe Tyr Tyr Asn Leu
  10                          1445                1450                1455

              CAG GAG ATT GAA GTA ATT AGT GTT ACT GGT GGG TAT TGG TCG AGT TAT 4416
              Gln Glu Ile Glu Val Ile Ser Val Thr Gly Gly Tyr Trp Ser Ser Tyr
                          1460                1465                1470
  15
              AAA ATA TCC AAC CCG ATT AAT ATC AAT ACG GGC ATT GAT AGT GCT AAA 4464
              Lys Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
                          1475                1480                1485

  20          GTA AAA GTC ACC GTA AAA GCG GGT GGT GAC GAT CAA ATC TTT ACT GCT 4512
              Val Lys Val Thr Val Lys Ala Gly Gly Asp Asp Gln Ile Phe Thr Ala
                  1490            1495                1500

              GAT AAT AGT ACC TAT GTT CCT CAG CAA CCG GCA CCC AGT TTT GAG GAG 4560
  25          Asp Asn Ser Thr Tyr Val Pro Gln Gln Pro Ala Pro Ser Phe Glu Glu
              1505                1510                1515            1520

              ATG ATT TAT CAG TTC AAT AAC CTG ACA ATA GAT TGT AAG AAT TTA AAT 4608
              Met Ile Tyr Gln Phe Asn Asn Leu Thr Ile Asp Cys Lys Asn Leu Asn
  30                          1525                1530                1535

              TTC ATC GAC AAT CAG GCA CAT ATT GAG ATT GAT TTC ACC GCT ACG GCA 4656
              Phe Ile Asp Asn Gln Ala His Ile Glu Ile Asp Phe Thr Ala Thr Ala
                          1540                1545                1550
  35
              CAA GAT GGC CGA TTC TTG GGT GCA GAA ACT TTT ATT ATC CCG GTA ACT 4704
              Gln Asp Gly Arg Phe Leu Gly Ala Glu Thr Phe Ile Ile Pro Val Thr
                      1555                1560                1565

  40          AAA AAA GTT CTC GGT ACT GAG AAC GTG ATT GCG TTA TAT AGC GAA AAT 4752
              Lys Lys Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn
                  1570                1575                1580

              AAC GGT GTT CAA TAT ATG CAA ATT GGC GCA TAT CGT ACC CGT TTG AAT 4800
  45          Asn Gly Val Gln Tyr Met Gln Ile Gly Ala Tyr Arg Thr Arg Leu Asn
              1585                1590                1595                1600

              ACG TTA TTC GCT CAA CAG TTG GTT AGC CGT GCT AAT CGT GGC ATT GAT 4848
              Thr Leu Phe Ala Gln Gln Leu Val Ser Arg Ala Asn Arg Gly Ile Asp
  50                          1605                1610                1615

              GCA GTG CTC AGT ATG GAA ACT CAG AAT ATT CAG GAA CCG CAA TTA GGA 4896
              Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly
                          1620                1625                1630
  55
              GCG GGC ACA TAT GTG CAG CTT GTG TTG GAT AAA TAT GAT GAG TCT ATT 4944
              Ala Gly Thr Tyr Val Gln Leu Val Leu Asp Lys Tyr Asp Glu Ser Ile
                      1635                1640                1645

  60          CAT GGC ACT AAT AAA AGC TTT GCT ATT GAA TAT GTT GAT ATA TTT AAA 4992
              His Gly Thr Asn Lys Ser Phe Ala Ile Glu Tyr Val Asp Ile Phe Lys
                  1650                1655                1660

              GAG AAC GAT AGT TTT GTG ATT TAT CAA GGA GAA CTT AGC GAA ACA AGT 5040
  65          Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser
              1665                1670                1675                1680

              CAA ACT GTT GTG AAA GTT TTC TTA TCC TAT TTT ATA GAG GCG ACT GGA 5088
              Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly
  70                          1685                1690                1695
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SUBSTITUTE SHEET (RULE 26)

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     AAT AAG AAC CAC TTA TGG GTA CGT GCT AAA TAC CAA AAG GAA ACG ACT 5136
     Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr
                     1700                1705                1710

 5   GAT AAG ATC TTG TTC GAC CGT ACT GAT GAG AAA GAT CCG CAC GGT TGG 5184
     Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp
                     1715                1720                1725

     TTT CTC AGC GAC GAT CAC AAG ACC TTT AGT GGT CTC TCT TCC GCA CAG 5232
10   Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln
             1730                1735                1740

     GCA TTA AAG AAC GAC AGT GAA CCG ATG GAT TTC TCT GGC GCC AAT GCT 5280
     Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala
15   1745                1750                1755                1760

     CTC TAT TTC TGG GAA CTG TTC TAT TAC ACG CCG ATG ATG ATG GCT CAT 5328
     Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His
                     1765                1770                1775
20
     CGT TTG TTG CAG GAA CAG AAT TTT GAT GCG GCG AAC CAT TGG TTC CGT 5376
     Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg
                 1780                1785                1790

25   TAT GTC TGG AGT CCA TCC GGT TAT ATC GTT GAT GGT AAA ATT GCT ATC 5424
     Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile
                     1795                1800                1805

     TAC CAC TGG AAC GTG CGA CCG CTG GAA GAA GAC ACC AGT TGG AAT GCA 5472
30   Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala
             1810                1815                1820

     CAA CAA CTG GAC TCC ACC GAT CCA GAT GCT GTA GCC CAA GAT GAT CCG 5520
     Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro
35   1825                1830                1835                1840

     ATG CAC TAC AAG GTG GCT ACC TTT ATG GCG ACG TTG GAT CTG CTA ATG 5568
     Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met
                     1845                1850                1855
40
     GCC CGT GGT GAT GCT GCT TAC CGC CAG TTA GAG CGT GAT ACG TTG GCT 5616
     Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala
                     1860                1865                1870

45   GAA GCT AAA ATG TGG TAT ACA CAG GCG CTT AAT CTG TTG GGT GAT GAG 5664
     Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu
                 1875                1880                1885

     CCA CAA GTG ATG CTG AGT ACG ACT TGG GCT AAT CCA ACA TTG GGT AAT 5712
50   Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn
             1890                1895                1900

     GCT GCT TCA AAA ACC ACA CAG CAG GTT CGT CAG CAA GTG CTT ACC CAG 5760
     Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln
55   1905                1910                1915                1920

     TTG CGT CTC AAT AGC AGG GTA AAA ACC CCG TTG CTA GGA ACA GCC AAT 5808
     Leu Arg Leu Asn Ser Arg Val Lys Thr Pro Leu Leu Gly Thr Ala Asn
                     1925                1930                1935
60
     TCC CTG ACC GCT TTA TTC CTG CCG CAG GAA AAT AGC AAG CTC AAA GGC 5856
     Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn Ser Lys Leu Lys Gly
                     1940                1945                1950

65   TAC TGG CGG ACA CTG GCG CAG CGT ATG TTT AAT TTA CGT CAT AAT CTG 5904
     Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn Leu Arg His Asn Leu
                 1955                1960                1965

     TCG ATT GAC GGC CAG CCG CTC TCC TTG CCG CTG TAT GCT AAA CCG GCT 5952
70   Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu Tyr Ala Lys Pro Ala
             1970                1975                1980
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      GAT CCA AAA GCT TTA CTG AGT GCG GCG GTT TCA GCT TCT CAA GGG GGA 6000
      Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser Ala Ser Gln Gly Gly
 5    1985           1990           1995           2000

      GCC GAC TTG CCG AAG GCG CCG CTG ACT ATT CAC CGC TTC CCT CAA ATG 6048
      Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His Arg Phe Pro Gln Met
                     2005           2010           2015

10    CTA GAA GGG GCA CGG GGC TTG GTT AAC CAG CTT ATA CAG TTC GGT AGT 6096
      Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu Ile Gln Phe Gly Ser
                     2020           2025           2030

      TCA CTA TTG GGG TAC AGT GAG CGT CAG GAT GCG GAA GCT ATG AGT CAA 6144
15    Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala Glu Ala Met Ser Gln
                     2035           2040           2045

      CTA CTG CAA ACC CAA GCC AGC GAG TTA ATA CTG ACC AGT ATT CGT ATG 6192
      Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu Thr Ser Ile Arg Met
20    2050           2055                2060

      CAG GAT AAC CAA TTG GCA GAG CTG GAT TCG GAA AAA ACC GCC TTG CAA 6240
      Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu Lys Thr Ala Leu Gln
      2065           2070           2075               2080
25
      GTC TCT TTA GCT GGA GTG CAA CAA CGG TTT GAC AGC TAT AGC CAA CTG 6288
      Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp Ser Tyr Ser Gln Leu
                     2085           2090           2095

30    TAT GAG GAG AAC ATC AAC GCA GGT GAG CAG CGA GCG CTG GCG TTA CGC 6336
      Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg Ala Leu Ala Leu Arg
                     2100           2105           2110

      TCA GAA TCT GCT ATT GAG TCT CAG GGA GCG CAG ATT TCC CGT ATG GCA 6384
35    Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln Ile Ser Arg Met Ala
                     2115           2120           2125

      GGC GCG GGT GTT GAT ATG GCA CCA AAT ATC TTC GGC CTG GCT GAT GGC 6432
      Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe Gly Leu Ala Asp Gly
40    2130           2135           2140

      GGC ATG CAT TAT GGT GCT ATT GCC TAT GCC ATC GCT GAC GGT ATT GAG 6480
      Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile Ala Asp Gly Ile Glu
      2145           2150           2155           2160
45
      TTG AGT GCT TCT GCC AAG ATG GTT GAT GCG GAG AAA GTT GCT CAG TCG 6528
      Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu Lys Val Ala Gln Ser
                     2165           2170           2175

50    GAA ATA TAT CGC CGT CGC CGT CAA GAA TGG AAA ATT CAG CGT GAC AAC 6576
      Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys Ile Gln Arg Asp Asn
                     2180           2185           2190

      GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA CTG GAA TCA CTG TCT 6624
55    Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser
                     2195           2200           2205

      ATT CGC CGT GAA GCC GCT GAA ATG CAA AAA GAG TAC CTG AAA ACC CAG 6672
      Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln
60    2210           2215           2220

      CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA AGA AGC AAA TTC AGT 6720
      Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser
      2225           2230           2235           2240
65
      AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT TTG TCA GGT ATT TAT 6768
      Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr
                     2245           2250           2255

70    TTC CAG TTC TAT GAC TTG GCC GTA TCA CGT TGC CTG ATG GCA GAG CAA 6816
      Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln
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SUBSTITUTE SHEET (RULE 26)

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                2260                    2265      .          2270
        TCC TAT CAA TGG GAA GCT AAT GAT AAT TCC ATT AGC TTT GTC AAA CCG 6864
        Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro
   5           2275                    2280                2285

        GGT GCA TGG CAA GGA ACT TAC GCC GGC TTA TTG TGT GGA GAA GCT TTG 6912
        Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu
  10           2290                    2295                2300

        ATA CAA AAT CTG GCA CAA ATG GAA GAG GCA TAT CTG AAA TGG GAA TCT 6960
        Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser
        2305                2310                    2315                2320

  15    CGC GCT TTG GAA GTA GAA CGC ACG GTT TCA TTG GCA GTG GTT TAT GAT 7008
        Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp
                     2325                    2330                2335

        TCA CTG GAA GGT AAT GAT CGT TTT AAT TTA GCG GAA CAA ATA CCT GCA 7056
  20    Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala
                     2340                    2345                2350

        TTA TTG GAT AAG GGG GAG GGA ACA GCA GGA ACT AAA GAA AAT GGG TTA 7104
        Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu
  25                 2355                    2360                2365

        TCA TTG GCT AAT GCT ATC CTG TCA GCT TCG GTC AAA TTG TCC GAC TTG 7152
        Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu
  30                 2370                    2375                2380

        AAA CTG GGA ACG GAT TAT CCA GAC AGT ATC GTT GGT AGC AAC AAG GTT 7200
        Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val
        2385                2390                    2395                2400

  35    CGT CGT ATT AAG CAA ATC AGT GTT TCG CTA CCT GCA TTG GTT GGG CCT 7248
        Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro
                     2405      .         2410                2415

        TAT CAG GAT GTT CAG GCT ATG CTC AGC TAT GGT GGC AGT ACT CAA TTG 7296
  40    Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu
                     2420                    2425                2430  .

        CCG AAA GGT TGT TCA GCG TTG GCT GTG TCT CAT GGT ACC AAT GAT AGT 7344
        Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser
  45             2435                    2440                2445

        GGT CAG TTC CAG TTG GAT TTC AAT GAC GGC AAA TAC CTG CCA TTT GAA 7392
        Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu
                 2450                    2455                2460
  50
        GGT ATT GCT CTT GAT GAT CAG GGT ACA CTG AAT CTT CAA TTT CCG AAT 7440
        Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn
        2465                2470                    2475                2480

  55    GCT ACC GAC AAG CAG AAA GCA ATA TTG CAA ACT ATG AGC GAT ATT ATT 7488
        Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile
                     2485                    2490                2495

        TTG CAT ATT CGT TAT ACC ATC CGT TAA                              7515
  60    Leu His Ile Arg Tyr Thr Ile Arg  *
                     2500                    2505
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SUBSTITUTE SHEET (RULE 26)

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 2504 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12 (TcbA protein):

```
Met Gln Asn Ser Leu Ser Ser Thr Ile Asp Thr Ile Cys Gln Lys Leu
 1           5               10              15

Gln Leu Thr Cys Pro Ala Glu Ile Ala Leu Tyr Pro Phe Asp Thr Phe
             20              25              30

Arg Glu Lys Thr Arg Gly Met Val Asn Trp Gly Glu Ala Lys Arg Ile
         35              40              45

Tyr Glu Ile Ala Gln Ala Glu Gln Asp Arg Asn Leu Leu His Glu Lys
     50              55              60

Arg Ile Phe Ala Tyr Ala Asn Pro Leu Leu Lys Asn Ala Val Arg Leu
 65              70              75              80

Gly Thr Arg Gln Met Leu Gly Phe Ile Gln Gly Tyr Ser Asp Leu Phe
             85              90              95

Gly Asn Arg Ala Asp Asn Tyr Ala Ala Pro Gly Ser Val Ala Ser Met
         100             105             110

Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asn
         115             120             125

Leu His Asp Ser Ser Ser Ile Tyr Tyr Leu Asp Lys Arg Arg Pro Asp
     130             135             140

Leu Ala Ser Leu Met Leu Ser Gln Lys Asn Met Asp Glu Glu Ile Ser
145             150             155             160

Thr Leu Ala Leu Ser Asn Glu Leu Cys Leu Ala Gly Ile Glu Thr Lys
             165             170             175

Thr Gly Lys Ser Gln Asp Glu Val Met Asp Met Leu Ser Thr Tyr Arg
         180             185             190

Leu Ser Gly Glu Thr Pro Tyr His His Ala Tyr Glu Thr Val Arg Glu
         195             200             205

Ile Val His Glu Arg Asp Pro Gly Phe Arg His Leu Ser Gln Ala Pro
     210             215             220

Ile Val Ala Ala Lys Leu Asp Pro Val Thr Leu Leu Gly Ile Ser Ser
     225             230             235             240

His Ile Ser Pro Glu Leu Tyr Asn Leu Leu Ile Glu Glu Ile Pro Glu
             245             250             255

Lys Asp Glu Ala Ala Leu Asp Thr Leu Tyr Lys Thr Asn Phe Gly Asp
         260             265             270

Ile Thr Thr Ala Gln Leu Met Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr
         275             280             285

Gly Val Ser Pro Glu Asp Ile Ala Tyr Val Thr Thr Ser Leu Ser His
     290             295             300

Val Gly Tyr Ser Ser Asp Ile Leu Val Ile Pro Leu Val Asp Gly Val
305             310             315             320
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        Gly Lys Met Glu Val Val Arg Val Thr Arg Thr Pro Ser Asp Asn Tyr
                    325             330             335

   5    Thr Ser Gln Thr Asn Tyr Ile Glu Leu Tyr Pro Gln Gly Gly Asp Asn
                    340             345             350

        Tyr Leu Ile Lys Tyr Asn Leu Ser Asn Ser Phe Gly Leu Asp Asp Phe
                    355             360             365

  10    Tyr Leu Gln Tyr Lys Asp Gly Ser Ala Asp Trp Thr Glu Ile Ala His
                    370             375             380

        Asn Pro Tyr Pro Asp Met Val Ile Asn Gln Lys Tyr Glu Ser Gln Ala
        385             390             395             400

  15    Thr Ile Lys Arg Ser Asp Ser Asp Asn Ile Leu Ser Ile Gly Leu Gln
                    405             410             415

        Arg Trp His Ser Gly Ser Tyr Asn Phe Ala Ala Ala Asn Phe Lys Ile
  20                420             425             430

        Asp Gln Tyr Ser Pro Lys Ala Phe Leu Leu Lys Met Asn Lys Ala Ile
                    435             440             445

  25    Arg Leu Leu Lys Ala Thr Gly Leu Ser Phe Ala Thr Leu Glu Arg Ile
            450             455             460

        Val Asp Ser Val Asn Ser Thr Lys Ser Ile Thr Val Glu Val Leu Asn
        465             470             475             480

  30    Lys Val Tyr Arg Val Lys Phe Tyr Ile Asp Arg Tyr Gly Ile Ser Glu
                    485             490             495

        Glu Thr Ala Ala Ile Leu Ala Asn Ile Asn Ile Ser Gln Gln Ala Val
  35                500             505             510

        Gly Asn Gln Leu Ser Gln Phe Glu Gln Leu Phe Asn His Pro Pro Leu
                    515             520             525

  40    Asn Gly Ile Arg Tyr Glu Ile Ser Glu Asp Asn Ser Lys His Leu Pro
                    530             535             540

        Asn Pro Asp Leu Asn Leu Lys Pro Asp Ser Thr Gly Asp Asp Gln Arg
        545             550             555             560

  45    Lys Ala Val Leu Lys Arg Ala Phe Gln Val Asn Ala Ser Glu Leu Tyr
                    565             570             575

        Gln Met Leu Leu Ile Thr Asp Arg Lys Glu Asp Gly Val Ile Lys Asn
  50                580             585             590

        Asn Leu Glu Asn Leu Ser Asp Leu Tyr Leu Val Ser Leu Leu Ala Gln
                    595             600             605

  55    Ile His Asn Leu Thr Ile Ala Glu Leu Asn Ile Leu Leu Val Ile Cys
                    610             615             620

        Gly Tyr Gly Asp Thr Asn Ile Tyr Gln Ile Thr Asp Asp Asn Leu Ala
        625             630             635             640

  60    Lys Ile Val Glu Thr Leu Leu Trp Ile Thr Gln Trp Leu Lys Thr Gln
                    645             650             655

        Lys Trp Thr Val Thr Asp Leu Phe Leu Met Thr Thr Ala Thr Tyr Ser
  65                660             665             670

        Thr Thr Leu Thr Pro Glu Ile Ser Asn Leu Thr Ala Thr Leu Ser Ser
                    675             680             685

  70    Thr Leu His Gly Lys Glu Ser Leu Ile Gly Glu Asp Leu Lys Arg Ala
                    690             695             700
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SUBSTITUTE SHEET (RULE 26)

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        Met Ala Pro Cys Phe Thr Ser Ala Leu His Leu Thr Ser Gln Glu Val
        705             710             715             720

  5     Ala Tyr Asp Leu Leu Leu Trp Ile Asp Gln Ile Gln Pro Ala Gln Ile
                    725             730             735

        Thr Val Asp Gly Phe Trp Glu Glu Val Gln Thr Thr Pro Thr Ser Leu
                    740             745             750
 10
        Lys Val Ile Thr Phe Ala Gln Val Leu Ala Gln Leu Ser Leu Ile Tyr
                    755             760             765

        Arg Arg Ile Gly Leu Ser Glu Thr Glu Leu Ser Leu Ile Val Thr Gln
 15         770             775             780

        Ser Ser Leu Leu Val Ala Gly Lys Ser Ile Leu Asp His Gly Leu Leu
        785             790             795             800

 20     Thr Leu Met Ala Leu Glu Gly Phe His Thr Trp Val Asn Gly Leu Gly
                    805             810             815

        Gln His Ala Ser Leu Ile Leu Ala Ala Leu Lys Asp Gly Ala Leu Thr
                    820             825             830
 25
        Val Thr Asp Val Ala Gln Ala Met Asn Lys Glu Glu Ser Leu Leu Gln
                    835             840             845

        Met Ala Ala Asn Gln Val Glu Lys Asp Leu Thr Lys Leu Thr Ser Trp
 30         850             855             860

        Thr Gln Ile Asp Ala Ile Leu Gln Trp Leu Gln Met Ser Ser Ala Leu
        865             870             875             880

 35     Ala Val Ser Pro Leu Asp Leu Ala Gly Met Met Ala Leu Lys Tyr Gly
                    885             890             895

        Ile Asp His Asn Tyr Ala Ala Trp Gln Ala Ala Ala Ala Leu Met
                    900             905             910
 40
        Ala Asp His Ala Asn Gln Ala Gln Lys Lys Leu Asp Glu Thr Phe Ser
            915             920             925

        Lys Ala Leu Cys Asn Tyr Tyr Ile Asn Ala Val Val Asp Ser Ala Ala
 45         930             935             940

        Gly Val Arg Asp Arg Asn Gly Leu Tyr Thr Tyr Leu Leu Ile Asp Asn
        945             950             955             960

 50     Gln Val Ser Ala Asp Val Ile Thr Ser Arg Ile Ala Glu Ala Ile Ala
                    965             970             975

        Gly Ile Gln Leu Tyr Val Asn Arg Ala Leu Asn Arg Asp Glu Gly Gln
                    980             985             990
 55
        Leu Ala Ser Asp Val Ser Thr Arg Gln Phe Phe Thr Asp Trp Glu Arg
            995             1000            1005

        Tyr Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr
 60         1010            1015            1020

        Tyr Pro Glu Asn Tyr Val Asp Pro Thr Gln Arg Ile Gly Gln Thr Lys
        1025            1030            1035            1040

 65     Met Met Asp Ala Leu Leu Gln Ser Ile Asn Gln Ser Gln Leu Asn Ala
                    1045            1050            1055

        Asp Thr Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Ser Phe Glu Gln
                    1060            1065            1070
 70
        Val Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Val Asn Val
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SUBSTITUTE SHEET (RULE 26)

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            1075                1080                1085
      Asp Gln Gly Leu Thr Tyr Phe Ile Gly Ile Asp Gln Ala Ala Pro Gly
 5        1090                1095                1100
      Thr Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Cys Glu Asn Gly Lys
          1105                1110                1115                1120
10    Phe Ala Ala Asn Ala Trp Gly Glu Trp Asn Lys Ile Thr Cys Ala Val
                   1125                1130                1135
      Asn Pro Trp Lys Asn Ile Ile Arg Pro Val Val Tyr Met Ser Arg Leu
               1140                1145                1150
15    Tyr Leu Leu Trp Leu Glu Gln Gln Ser Lys Lys Ser Asp Asp Gly Lys
               1155                1160                1165
      Thr Thr Ile Tyr Gln Tyr Asn Leu Lys Leu Ala His Ile Arg Tyr Asp
          1170                1175                1180
20    Gly Ser Trp Asn Thr Pro Phe Thr Phe Asp Val Thr Glu Lys Val Lys
          1185                1190                1195                1200
      Asn Tyr Thr Ser Ser Thr Asp Ala Ala Glu Ser Leu Gly Leu Tyr Cys
                   1205                1210                1215
25    Thr Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Ser Met
               1220                1225                1230
      Gln Ser Ser Tyr Ser Ser Tyr Thr Asp Asn Asn Ala Pro Val Thr Gly
30             1235                1240                1245
      Leu Tyr Ile Phe Ala Asp Met Ser Ser Asp Asn Met Thr Asn Ala Gln
               1250                1255                1260
35    Ala Thr Asn Tyr Trp Asn Asn Ser Tyr Pro Gln Phe Asp Thr Val Met
          1265                1270                1275                1280
      Ala Asp Pro Asp Ser Asp Asn Lys Lys Val Ile Thr Arg Arg Val Asn
40                 1285                1290                1295
      Asn Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Thr Ser Asn
               1300                1305                1310
45    Ser Asn Tyr Ser Trp Gly Asp His Ser Leu Thr Met Leu Tyr Gly Gly
               1315                1320                1325
      Ser Val Pro Asn Ile Thr Phe Glu Ser Ala Ala Glu Asp Leu Arg Leu
               1330                1335                1340
50    Ser Thr Asn Met Ala Leu Ser Ile Ile His Asn Gly Tyr Ala Gly Thr
          1345                1350                1355                1360
      Arg Arg Ile Gln Cys Asn Leu Met Lys Gln Tyr Ala Ser Leu Gly Asp
55                 1365                1370                1375
      Lys Phe Ile Ile Tyr Asp Ser Ser Phe Asp Asp Ala Asn Arg Phe Asn
               1380                1385                1390
60    Leu Val Pro Leu Phe Lys Phe Gly Lys Asp Glu Asn Ser Asp Asp Ser
               1395                1400                1405
      Ile Cys Ile Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr
65        1410                1415                1420
      Phe Ser Ser Lys Asp Asp Asn Lys Thr Ala Asp Tyr Asn Gly Gly Thr
      1425                1430                1435                1440
      Gln Cys Ile Asp Ala Gly Thr Ser Asn Lys Asp Phe Tyr Tyr Asn Leu
70             1445                1450                1455
```

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```
        Gln Glu Ile Glu Val Ile Ser Val Thr Gly Gly Tyr Trp Ser Ser Tyr
                      1460                1465            1470

  5     Lys Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
                      1475                1480                1485

        Val Lys Val Thr Val Lys Ala Gly Gly Asp Asp Gln Ile Phe Thr Ala
                1490                1495                1500

 10     Asp Asn Ser Thr Tyr Val Pro Gln Gln Pro Ala Pro Ser Phe Glu Glu
                1505                1510                1515                1520

        Met Ile Tyr Gln Phe Asn Asn Leu Thr Ile Asp Cys Lys Asn Leu Asn
                          1525                1530                1535

 15     Phe Ile Asp Asn Gln Ala His Ile Glu Ile Asp Phe Thr Ala Thr Ala
                      1540                1545                1550

        Gln Asp Gly Arg Phe Leu Gly Ala Glu Thr Phe Ile Ile Pro Val Thr
                      1555                1560                1565

 20     Lys Lys Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn
                1570                1575                1580

        Asn Gly Val Gln Tyr Met Gln Ile Gly Ala Tyr Arg Thr Arg Leu Asn
 25     1585                1590                1595                1600

        Thr Leu Phe Ala Gln Gln Leu Val Ser Arg Ala Asn Arg Gly Ile Asp
                          1605                1610                1615
 30
        Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly
                1620                1625                1630

        Ala Gly Thr Tyr Val Gln Leu Val Leu Asp Lys Tyr Asp Glu Ser Ile
 35             1635                1640                1645

        His Gly Thr Asn Lys Ser Phe Ala Ile Glu Tyr Val Asp Ile Phe Lys
                1650                1655                1660

 40     Glu Asn Asp Ser Phe Val Ile Tyr Gln Gly Glu Leu Ser Glu Thr Ser
        1665                1670                1675                1680

        Gln Thr Val Val Lys Val Phe Leu Ser Tyr Phe Ile Glu Ala Thr Gly
                          1685                1690                1695
 45
        Asn Lys Asn His Leu Trp Val Arg Ala Lys Tyr Gln Lys Glu Thr Thr
                      1700                1705                1710

 50     Asp Lys Ile Leu Phe Asp Arg Thr Asp Glu Lys Asp Pro His Gly Trp
                1715                1720                1725

        Phe Leu Ser Asp Asp His Lys Thr Phe Ser Gly Leu Ser Ser Ala Gln
                1730                1735                1740

 55     Ala Leu Lys Asn Asp Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ala
        1745                1750                1755                1760

        Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro Met Met Met Ala His
                          1765                1770                1775
 60
        Arg Leu Leu Gln Glu Gln Asn Phe Asp Ala Ala Asn His Trp Phe Arg
                      1780                1785                1790

 65     Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val Asp Gly Lys Ile Ala Ile
                      1795                1800                1805

        Tyr His Trp Asn Val Arg Pro Leu Glu Glu Asp Thr Ser Trp Asn Ala
                1810                1815                1820

 70     Gln Gln Leu Asp Ser Thr Asp Pro Asp Ala Val Ala Gln Asp Asp Pro
        1825                1830                1835                1840
```

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Met His Tyr Lys Val Ala Thr Phe Met Ala Thr Leu Asp Leu Leu Met
             1845   1850   1855

5   Ala Arg Gly Asp Ala Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Ala
             1860   1865   1870

     Glu Ala Lys Met Trp Tyr Thr Gln Ala Leu Asn Leu Leu Gly Asp Glu
             1875   1880   1885
10
     Pro Gln Val Met Leu Ser Thr Thr Trp Ala Asn Pro Thr Leu Gly Asn
             1890   1895   1900

     Ala Ala Ser Lys Thr Thr Gln Gln Val Arg Gln Gln Val Leu Thr Gln
15   1905   1910   1915   1920

     Leu Arg Leu Asn Ser Arg Val Lys Thr Pro Leu Leu Gly Thr Ala Asn
             1925   1930   1935

20   Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn Ser Lys Leu Lys Gly
             1940   1945   1950

     Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn Leu Arg His Asn Leu
             1955   1960   1965
25
     Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu Tyr Ala Lys Pro Ala
             1970   1975   1980

     Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser Ala Ser Gln Gly Gly
30   1985   1990   1995   2000

     Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His Arg Phe Pro Gln Met
             2005   2010   2015

35   Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu Ile Gln Phe Gly Ser
             2020   2025   2030

     Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala Glu Ala Met Ser Gln
             2035   2040   2045
40
     Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu Thr Ser Ile Arg Met
             2050   2055   2060

     Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu Lys Thr Ala Leu Gln
45   2065   2070   2075   2080

     Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp Ser Tyr Ser Gln Leu
             2085   2090   2095

50   Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg Ala Leu Ala Leu Arg
             2100   2105   2110

     Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln Ile Ser Arg Met Ala
             2115   2120   2125
55
     Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe Gly Leu Ala Asp Gly
             2130   2135   2140

     Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile Ala Asp Gly Ile Glu
60   2145   2150   2155   2160

     Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu Lys Val Ala Gln Ser
             2165   2170   2175

65   Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys Ile Gln Arg Asp Asn
             2180   2185   2190

     Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln Leu Glu Ser Leu Ser
             2195   2200   2205
70
     Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu Tyr Leu Lys Thr Gln

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```
            2210              2215                  2220
       Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu Arg Ser Lys Phe Ser
       2225              2230              2235              2240
 5
       Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser Gly Ile Tyr
                     2245              2250              2255

10     Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met Ala Glu Gln
                     2260              2265              2270

       Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile Ser Phe Val Lys Pro
                 2275              2280              2285

15     Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu Cys Gly Glu Ala Leu
                 2290              2295              2300

       Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr Leu Lys Trp Glu Ser
       2305              2310              2315              2320
20
       Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Val Val Tyr Asp
                 2325              2330              2335

       Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala Glu Gln Ile Pro Ala
25               2340              2345              2350

       Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr Lys Glu Asn Gly Leu
                 2355              2360              2365

30     Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val Lys Leu Ser Asp Leu
                 2370              2375              2380

       Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val Gly Ser Asn Lys Val
       2385              2390              2395              2400
35
       Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro Ala Leu Val Gly Pro
                 2405              2410              2415

       Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly Gly Ser Thr Gln Leu
40               2420              2425              2430

       Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His Gly Thr Asn Asp Ser
                 2435              2440              2445

45     Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys Tyr Leu Pro Phe Glu
                 2450              2455              2460

       Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn Leu Gln Phe Pro Asn
       2465              2470              2475              2480
50
       Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr Met Ser Asp Ile Ile
                 2485              2490              2495

       Leu His Ile Arg Tyr Thr Ile Arg *
55               2500              2505
```

   (2) INFORMATION FOR SEQ ID NO:13:

60   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 12 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
65   (ii) MOLECULE TYPE: peptide

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13 (TcdA$_{ii}$ N-terminus):

         Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Xaa Ala

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```
              1                5             .    10
```

(2) INFORMATION FOR SEQ ID NO:14:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 12 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14 (TcdB N-terminus):

        Met Gln Asn Ser Gln Thr Phe Ser Val Gly Glu Leu
        1   5   10

(2) INFORMATION FOR SEQ ID NO:15:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 14 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15 (TcaA$_{ii}$ N-terminus):

        Ala Gln Asp Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr
        1   5   10

(2) INFORMATION FOR SEQ ID NO:16:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16 (TcbA N-terminus):

        Met Gln Asn Ser Leu
        1   5

(2) INFORMATION FOR SEQ ID NO:17:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 10 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17 (TcdA$_{ii}$-PT111 internal
peptide):

        Ala Phe Asn Ile Asp Asp Val Ser Leu Phe
        1   5   10

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(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18 ($TcdA_{ii}$- PT79 internal peptide):

```
Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
1               5                   10              15
```

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19 ($TcaB_i$- PT158 internal peptide):

```
Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile Gly Ser
1               5                   10              15

Leu Gln Leu Phe Ile
            20
```

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20 ($TcaB_i$- PT 108 internal peptide):

```
Met Tyr Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21 ($TcbA_{ii}$- PT103 internal peptide):

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```
        Gly Ile Asp Ala Val Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro
        1               5                  10       ___            15

        Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
                    20              25
```

(2) INFORMATION FOR SEQ ID NO:22:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 15 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22 (TcbA$_{ii}$- PT56 internal
peptide):

```
        Ile Ser Asn Pro Ile Asn Ile Asn Thr Gly Ile Asp Ser Ala Lys
        1               5                  10               15
```

(2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 13 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23 (TcbA- PT81 (a)
internal peptide):

```
        Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys
        1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:24:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 22 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24 (TcbA$_{ii}$- PT81 (b)
internal peptide):

```
        Val Leu Gly Thr Glu Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly
        1               5                  10               15

        Val Gln Tyr Met Gln Ile
                    20
```

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(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6054 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: DNA (genomic)
   (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..43
        (D) OTHER INFORMATION: /product= "end of TcaA$_{iii}$"
   (ix) FEATURE:
        (A) NAME/KEY: RBS
        (B) LOCATION: 51 58
   (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 65 3634
        (D) OTHER INFORMATION: /product= "TcaB$_i$"

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
A GTA GCC CAA AAC TTA AGT GCC GCA ATC AGC AAT CGT CAG TAACCGGATA   50
  Val Ala Gln Asn Leu Ser Ala Ala Ile Ser Asn Arg Gln •••


AAGAAGGAAT TGATT ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA  100
                  Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu
                   1                5                   10

GCG CGC CGT GAT GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC   148
Ala Arg Arg Asp Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro
             15              20                  25

GCA GAT TTA AAA GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT   196
Ala Asp Leu Lys Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr
         30              35              40

CTG TTG CTG GAT ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG   244
Leu Leu Leu Asp Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu
 45              50              55              60

TCC GAA GCG ATT GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG   292
Ser Glu Ala Ile Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu
             65              70                  75

GGC TAT GAC GGC ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT   340
Gly Tyr Asp Gly Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp
             80              85                  90

GAA CAG TTT TTA TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT   388
Glu Gln Phe Leu Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr
             95              100                 105

TGG GCT GGC AAG GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT   436
Trp Ala Gly Lys Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp
110                 115                 120

CCA ACA TTG CGA TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA   484
Pro Thr Leu Arg Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln
125                 130                 135                 140

GGT ATT TCT CAA GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA   532
Gly Ile Ser Gln Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu
                145                 150                 155

CGT GAT TAT CTA ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT   580
```

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```
        Arg Asp Tyr Leu Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile
                160             165             170

  5     ACT GCC TGC CAA GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT   628
        Thr Ala Cys Gln Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg
                175             180             185

        ACA CAG AAT GCA CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC   676
        Thr Gln Asn Ala Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val
 10             190             195             200

        ACT GAT GGC GGT AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA   724
        Thr Asp Gly Gly Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala
        205             210             215             220
 15
        ATT AAT GCC GGG ATT AGT GAG GCA TAT TCA GGG CAT GTC GAG CCT TTC   772
        Ile Asn Ala Gly Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe
                        225             230             235

 20     TGG GAA AAT AAC AAG CTG CAC ATC CGT TGG TTT ACT ATC TCG AAA GAA   820
        Trp Glu Asn Asn Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu
                        240             245             250

        GAT AAA ATA GAT TTT GTT TAT AAA AAC ATC TGG GTG ATG AGT AGC GAT   868
 25     Asp Lys Ile Asp Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp
                255             260             265

        TAT AGC TGG GCA TCA AAG AAA AAA ATC TTG GAA CTT TCT TTT ACT GAC   916
        Tyr Ser Trp Ala Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp
 30             270             275             280

        TAC AAT AGA GTT GGA GCA ACA GGA TCA TCA AGC CCG ACT GAA GTA GCT   964
        Tyr Asn Arg Val Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala
        285             290             295             300
 35
        TCA CAA TAT GGT TCT GAT GCT CAG ATG AAT ATT TCT GAT GAT GGG ACT  1012
        Ser Gln Tyr Gly Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr
                        305             310             315

 40     GTA CTT ATT TTT CAG AAT GCC GGC GGA GCT ACT CCC AGT ACT GGA GTG  1060
        Val Leu Ile Phe Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val
                320             325             330

        ACG TTA TGT TAT GAC TCT GGC AAC GTG ATT AAG AAC CTA TCT AGT ACA  1108
 45     Thr Leu Cys Tyr Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr
                335             340             345

        GGA AGT GCA AAT TTA TCG TCA AAG GAT TAT GCC ACA ACT AAA TTA CGC  1156
        Gly Ser Ala Asn Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg
 50             350             355             360

        ATG TGT CAT GGA CAA AGT TAC AAT GAT AAT AAC TAC TGC AAT TTT ACA  1204
        Met Cys His Gly Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr
        365             370             375             380
 55
        CTC TCT ATT AAT ACA ATA GAA TTC ACC TCC TAC GGC ACA TTC TCA TCA  1252
        Leu Ser Ile Asn Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser
                        385             390             395

 60     GAT GGA AAA CAA TTT ACA CCA CCT TCT GGT TCT GCC ATT GAT TTA CAC  1300
        Asp Gly Lys Gln Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His
                400             405             410

        CTC CCT AAT TAT GTA GAT CTC AAC GCG CTA TTA GAT ATT AGC CTC GAT  1348
 65     Leu Pro Asn Tyr Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp
                415             420             425

        TCA CTA CTT AAT TAT GAC GTT CAG GGG CAG TTT GGC GGA TCT AAT CCG  1396
        Ser Leu Leu Asn Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro
 70             430             435             440
```

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GTT GAT AAT TTC AGT GGT CCC TAT GGT ATT TAT CTA TGG GAA ATC TTC 1444
Val Asp Asn Phe Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe
445   450   455   460

5   TTC CAT ATT CCG TTC CTT GTT ACG GTC CGT ATG CAA ACC GAA CAA CGT 1492
    Phe His Ile Pro Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg
                    465   470   475

    TAC GAA GAC GCG GAC ACT TGG TAC AAA TAT ATT TTC CGC AGC GCC GGT 1540
10 Tyr Glu Asp Ala Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly
                    480   485   490

    TAT CGC GAT GCT AAT GGC CAG CTC ATT ATG GAT GGC AGT AAA CCA CGT 1588
    Tyr Arg Asp Ala Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg
15   495   500   505

    TAT TGG AAT GTG ATG CCA TTG CAA CTG GAT ACC GCA TGG GAT ACC ACA 1636
    Tyr Trp Asn Val Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr
    510   515   520
20
    CAG CCC GCC ACC ACT GAT CCA GAT GTG ATC GCT ATG GCG GAC CCG ATG 1684
    Gln Pro Ala Thr Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met
    525   530   535   540

25 CAT TAC AAG CTG GCG ATA TTC CTG CAT ACC CTT GAT CTA TTG ATT GCC 1732
    His Tyr Lys Leu Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala
                    545   550   555

    CGA GGC GAC AGC GCT TAC CGT CAA CTT GAA CGC GAT ACT CTA GTC GAA 1780
30 Arg Gly Asp Ser Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu
                    560   565   570

    GCC AAA ATG TAC TAC ATT CAG GCA CAA CAG CTA CTG GGA CCG CGC CCT 1828
    Ala Lys Met Tyr Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro
35   575   580   585

    GAT ATC CAT ACC ACC AAT ACT TGG CCA AAT CCC ACC TTG AGT AAA GAA 1876
    Asp Ile His Thr Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu
                    590   595   600
40
    GCT GGC GCT ATT GCC ACA CCG ACA TTC CTC AGT TCA CCG GAG GTG ATG 1924
    Ala Gly Ala Ile Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met
    605   610   615   620

45 ACG TTC GCT GCC TGG CTA AGC GCA GGC GAT ACC GCA AAT ATT GGC GAC 1972
    Thr Phe Ala Ala Trp Leu Ser Ala Gly Asp Thr Ala Asn Ile Gly Asp
                    625   630   635

    GGT GAT TTC TTG CCA CCG TAC AAC GAT GTA CTA CTC GGT TAC TGG GAT 2020
50 Gly Asp Phe Leu Pro Pro Tyr Asn Asp Val Leu Leu Gly Tyr Trp Asp
                    640   645   650

    AAA CTT GAG TTA CGC CTA TAC AAC CTG CGC CAC AAT CTG AGT CTG GAT 2068
    Lys Leu Glu Leu Arg Leu Tyr Asn Leu Arg His Asn Leu Ser Leu Asp
55   655   660   665

    GGT CAA CCG CTA AAT CTG CCA CTG TAT GCC ACG CCG GTA GAC CCG AAA 2116
    Gly Gln Pro Leu Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys
                    670   675   680
60
    ACC CTG CAA CGC CAG CAA GCC GGA GGG GAC GGT ACA GGC AGT AGT CCG 2164
    Thr Leu Gln Arg Gln Gln Ala Gly Gly Asp Gly Thr Gly Ser Ser Pro
    685   690   695   700

65 GCT GGT GGT CAA GGC AGT GTT CAG GGC TGG CGC TAT CCG TTA TTG GTA 2212
    Ala Gly Gly Gln Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val
                    705   710   715

    GAA CGC GCC CGC TCT GCC GTG AGT TTG TTG ACT CAG TTC GGC AAC AGC 2260
70 Glu Arg Ala Arg Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser
                    720   725   730

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SUBSTITUTE SHEET (RULE 26)

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        TTA CAA ACA ACG TTA GAA CAT CAG GAT AAT GAA AAA ATG ACG ATA CTG 2308
        Leu Gln Thr Thr Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu
                735                     740                 745
  5
        TTG CAG ACT CAA CAG GAA GCC ATC CTG AAA CAT CAG CAC GAT ATA CAA 2356
        Leu Gln Thr Gln Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln
                750                 755                 760
 10     CAA AAT AAT CTA AAA GGA TTA CAA CAC AGC CTG ACC GCA TTA CAG GCT 2404
        Gln Asn Asn Leu Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala
        765                 770                 775                 780

        AGC CGT GAT GGC GAC ACA TTG CGG CAA AAA CAT TAC AGC GAC CTG ATT 2452
        Ser Arg Asp Gly Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile
 15                         785                 790                 795

        AAC GGT GGT CTA TCT GCG GCA GAA ATC GCC GGT CTG ACA CTA CGC AGC 2500
        Asn Gly Gly Leu Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser
 20                     800                 805                 810

        ACC GCC ATG ATT ACC AAT GGC GTT GCA ACG GGA TTG CTG ATT GCC GGC 2548
        Thr Ala Met Ile Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly
                815                 820                 825
 25
        GGA ATC GCC AAC GCG GTA CCT AAC GTC TTC GGG CTG GCT AAC GGT GGA 2596
        Gly Ile Ala Asn Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly
                830                 835                 840

 30     TCG GAA TGG GGA GCG CCA TTA ATT GGC TCC GGG CAA GCA ACC CAA GTT 2644
        Ser Glu Trp Gly Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val
        845                 850                 855                 860

        GGC GCC GGC ATC CAG GAT CAG AGC GCG GGC ATT TCA GAA GTG ACA GCA 2692
        Gly Ala Gly Ile Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala
 35                     865                 870                 875

        GGC TAT CAG CGT CGT CAG GAA GAA TGG GCA TTG CAA CGG GAT ATT GCT 2740
        Gly Tyr Gln Arg Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala
 40                     880                 885                 890

        GAT AAC GAA ATA ACC CAA CTG GAT GCC CAG ATA CAA AGC CTG CAA GAG 2788
        Asp Asn Glu Ile Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu
                895                 900                 905
 45
        CAA ATC ACG ATG GCA CAA AAA CAG ATC ACG CTC TCT GAA ACC GAA CAA 2836
        Gln Ile Thr Met Ala Gln Lys Gln Ile Thr Leu Ser Glu Thr Glu Gln
                910                 915                 920

 50     GCG AAT GCC CAA GCG ATT TAT GAC CTG CAA ACC ACT CGT TTT ACC GGG 2884
        Ala Asn Ala Gln Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly
        925                 930                 935                 940

        CAG GCA CTG TAT AAC TGG ATG GCC GGT CGT CTC TCC GCG CTC TAT TAC 2932
        Gln Ala Leu Tyr Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr
 55                     945                 950                 955

        CAA ATG TAT GAT TCC ACT CTG CCA ATC TGT CTC CAG CCA AAA GCC GCA 2980
        Gln Met Tyr Asp Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala
 60                     960                 965                 970

        TTA GTA CAG GAA TTA GGC GAG AAA GAG AGC GAC AGT CTT TTC CAG GTT 3028
        Leu Val Gln Glu Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val
                975                 980                 985
 65
        CCG GTG TGG AAT GAT CTG TGG CAA GGG CTG TTA GCA GGA GAA GGT TTA 3076
        Pro Val Trp Asn Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu
                990                 995                 1000

 70     AGT TCA GAG CTA CAG AAA CTG GAT GCC ATC TGG CTT GCA CGT GGT GGT 3124
        Ser Ser Glu Leu Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly
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SUBSTITUTE SHEET (RULE 26)

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                1005                 1010            .1015                 1020
        ATT GGG CTA GAA GCC ATC CGC ACC GTG TCG CTG GAT ACC CTG TTT GGC 3172
        Ile Gly Leu Glu Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly
  5                          1025                 1030                 1035

        ACA GGG ACG TTA AGT GAA AAT ATC AAT AAA GTG CTT AAC GGG GAA ACG 3220
        Thr Gly Thr Leu Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr
                             1040                 1045                 1050
 10
        GTA TCT CCA TCC GGT GGC GTC ACT CTG GCG CTG ACA GGG GAT ATC TTC 3268
        Val Ser Pro Ser Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe
                             1055                 1060                 1065

 15     CAA GCA ACA CTG GAT TTG AGT CAG CTA GGT TTG GAT AAC TCT TAC AAC 3316
        Gln Ala Thr Leu Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn
                        1070                 1075                 1080

        TTG GGT AAC GAG AAG AAA CGT CGT ATT AAA CGT ATC GCC GTC ACC CTG 3364
 20     Leu Gly Asn Glu Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu
        1085                 1090                 1095                 1100

        CCA ACA CTT CTG GGG CCA TAT CAA GAT CTT GAA GCC ACA CTG GTA ATG 3412
        Pro Thr Leu Leu Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met
 25                          1105                 1110                 1115

        GGT GCG GAA ATC GCC GCC TTA TCA CAC GGT GTG AAT GAC GGA GGC CGG 3460
        Gly Ala Glu Ile Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg
                             1120                 1125                 1130
 30
        TTT GTT ACC GAC TTT AAC GAC AGC CGT TTT CTG CCT TTT GAA GGT CGA 3508
        Phe Val Thr Asp Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg
                             1135                 1140                 1145

 35     GAT GCA ACA ACC GGC ACA CTG GAG CTC AAT ATT TTC CAT GCG GGT AAA 3556
        Asp Ala Thr Thr Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys
                        1150                 1155                 1160

        GAG GGA ACG CAA CAC GAG TTG GTC GCG AAT CTG AGT GAC ATC ATT GTG 3604
 40     Glu Gly Thr Gln His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val
        1165                 1170                 1175                 1180

        CAT CTG AAT TAC ATC ATT CGA GAC GCG TAA ATTTCTTTTC TTTGTCGATT    3654
        His Leu Asn Tyr Ile Ile Arg Asp Ala  *
 45                     1185                 1190

        ACAGGTCCCT ATCAGGGGCC TGTTATTAAG GAGTACTTTA TGCAGGATTC ACCAGAAGTA 3714

        TCGATTACAA CGCTGTCACT TCCCAAAGGT GGCGGTGCTA TCAATGGCAT GGGAGAAGCA 3774
 50
        CTGAATGCTG CCGGCCCTGA TGGAATGGCC TCCCTATCTC TGCCATTACC CCTTTCGACC 3834

        GGCAGAGGGA CGGCTCCTGG ATTATCGCTG ATTTACAGCA ACAGTGCAGG TAATGGGCCT 3894

 55     TTCGGCATCG GCTGGCAATG CGGTGTTATG TCCATTAGCC GACGCACCCA ACATGGCATT 3954

        CCACAATACG GTAATGACGA CACGTTCCTA TCCCCACAAG GCGAGGTCAT GAATATCGCC 4014

        CTGAATGACC AAGGGCAACC TGATATCCGT CAAGACGTTA AAACGCTGCA AGGCGTTACC 4074
 60
        TTGCCAATTT CCTATACCGT GACCCGCTAT CAAGCCCGCC AGATCCTGGA TTTCAGTAAA 4134

        ATCGAATACT GGCAACCTGC CTCCGGTCAA GAAGGACGCG CTTTCTGGCT GATATCGACA 4194

 65     CCGGACGGGC ATCTACACAT CTTAGGGAAA ACCGCGCAGG CTTGTCTGGC AAATCCGCAA 4254

        AATGACCAAC AAATCGCCCA GTGGTTGCTG GAAGAAACTG TGACGCCAGC CGGTGAACAT 4314

        GTCAGCTATC AATATCGAGC CGAAGATGAA GCCCATTGTG ACGACAATGA AAAAACCGCT 4374
 70
        CATCCCAATG TTACCGCACA GCGCTATCTG GTACAGGTGA ACTACAGGCA ACATCAAACC 4434
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SUBSTITUTE SHEET (RULE 26)

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        ACAAGCCAGC CTGTTCGTAC TGGATAACGC ACCTCCCGCA CCGGAAGAGT GGCTGTTTCA 4494

5       TCTGGTCTTT GACCACGGTG AGCGCGTACC TCACTTCATA CCGTGCCAAC ATGGGATGCA 4554

        GGTACAGCGC AATGGTCTGT ACGCCCGGAT ATCTTCTCTC GCTATGAATA TGGTTTTGAA 4614

10      GTGCGTACTC GCCGCTTATG TCAACAAGTG CTGATGTTTC ACCGCACCGC GCTCATGGCC 4674

        GGAGAAGCCA GTACCAATGA CGCCCCGGAA CTGGTTGGAC GCTTAATACT GGAATATGAC 4634

        AAAAACGCCA GCGTCACCAC GTTGATTACC ATCCGTCAAT TAAGCCATGA ATCGGACGGG 4794

15      AGGCCAGTCA CCCAGCCACC ACTAGAACTA GCCTGGCAAC GGTTTGATCT GGAGAAAATC 4854

        CCGACATGGC AACGCTTTGA CGCACTAGAT AATTTTAACT CGCAGCAACG TTATCAACTG 4865

        GTTGATCTGC GGGGAGAAGG GTTGCCAGGT ATGCTGTATC AAGATCGAGG CGCTTGGTGG 4914

20      TATAAAGCTC CGCAACGTCA GGAAGACGGA GACAGCAATG CCGTCACTTA CGACAAAATC 4974

        GCCCCACTGC CTACCCTACC CAATTTGCAG GATAATGCCT CATTGATGGA TATCAACGGA 5034

        GACGGCCAAC TGGATTGGGT TGTTACCGCC TCCGGTATTC GCGGATACCA TAGTCAGCAA 5094

25      CCCGATGGAA AGTGGACGCA CTTTACGCCA ATCAATGCCT TGCCCGTGGA ATATTTTCAT 5214

        CCAAGCATCC AGTTCGCTGA CCTTACCGGG GCAGGCTTAT CTGATTTAGT GTTGATCGGG 5274

30      CCGAAAAGCG TGCGTCTATA TGCCAACCAG CGAAACGGCT GGCGTAAAGG AGAAGATGTC 5334

        CCCCAATCCA CAGGTATCAC CCTGCCTGTC ACAGGGACCG ATGCCCGCAA ACTGGTGGCT 5394

        TTCAGTGATA TGCTCGGTTC CGGTCAACAA CATCTGGTGG AAATCAAGGG TAATCGCGTC 5454

35      ACCTGTTGGC CGAATCTAGG GCATGGCCGT TTCGGTCAAC CACTAACTCT GTCAGGATTT 5514

        AGCCAGCCCG AAAATAGCTT CAATCCCGAA CGGCTGTTTC TGGCGGATAT CGACGGCTCC 5574

40      GGCACCACCG ACCTTATCTA TGCGCAATCC GGCTCTTTGC TCATTTATCT CAACCAAAGT 5634

        GGTAATCAGT TTGATGCCCC GTTGACATTA GCGTTGCCAG AAGGCGTACA ATTTGACAAC 5694

        ACTTGCCAAC TTCAAGTCGC CGATATTCAG GGATTAGGGA TAGCCAGCTT GATTCTGACT 5754

45      GTGCCACATA TCGCGCCACA TCACTGGCGT TGTGACCTGT CACTGACCAA ACCCTGGTTG 5814

        TTGAATGTAA TGAACAATAA CCGGGGCGCA CATCACACGC TACATTATCG TAGTTCCGCG 5874

50      CAATTCTGGT TGGATGAAAA ATTACAGCTC ACCAAAGCAG GCAAATCTCC GGCTTGTTAT 5934

        CTGCCGTTTC CAATGCATTT GCTATGGTAT ACCGAAATTC AGGATGAAAT CAGCGGCAAC 5994

55      CGGCTCACCA GTGAAGTCAA CTACAGCCAC GGCGTCTGGG ATGGTAAAGA GCGGGAATTC 6054
```

    (2) INFORMATION FOR SEQ ID NO:26:

60   (i)   SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 1189 amino acids
                  (B) TYPE: amino acid
                  (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: protein

65   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26 (TcaB protein):

        Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
        1   5   10   15

SUBSTITUTE SHEET (RULE 26)

```
      Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
                  20              25              30

 5    Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
                  35              40              45

      Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
              50              55              60

10    Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
          65              70              75              80

      Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
                      85              90              95

15    Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
                  100             105             110

      Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
20            115             120             125

      Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
          130             135             140

25    Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
      145             150             155             160

      Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
                      165             170             175

30    Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
                  180             185             190

      Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
35            195             200             205

      Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
          210             215             220

40    Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
      225             230             235             240

      Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Ile Asp
                      245             250             255

45    Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
                  260             265             270

      Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
50            275             280             285

      Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
          290             295             300

55    Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
      305             310             315             320

      Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
                  325             330             335

60    Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
                  340             345             350

      Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
65            355             360             365

      Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
                  370             375             380

70    Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
      385             390             395             400
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SUBSTITUTE SHEET (RULE 26)

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      Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
                      405             410             415

 5    Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn
                  420             425             430

      Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
              435             440             445

10    Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
          450             455             460

      Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
15    465             470             475             480

      Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                  485             490             495

20    Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
                  500             505             510

      Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
              515             520             525
25
      Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
          530             535             540

      Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
30    545             550             555             560

      Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
                  565             570             575

35    Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
                  580             585             590

      Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
              595             600             605
40
      Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
          610             615             620

      Trp Leu Ser Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu
45    625             630             635             640

      Pro Pro Tyr Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu
                  645             650             655

50    Arg Leu Tyr Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu
                  660             665             670

      Asn Leu Pro Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg
              675             680             685
55
      Gln Gln Ala Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln
          690             695             700

      Gly Ser Val Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg
60    705             710             715             720

      Ser Ala Val Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr
                  725             730             735

65    Leu Glu His Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln
                  740             745             750

      Gln Glu Ala Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu
              755             760             765
70
      Lys Gly Leu Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly
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SUBSTITUTE SHEET (RULE 26)

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              770                  775              780
        Asp Thr Leu Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu
        785             790             795             800
  5
        Ser Ala Ala Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile
                        805             810             815

 10     Thr Asn Gly Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn
                    820             825             830

        Ala Val Pro Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly
                835             840             845

 15     Ala Pro Leu Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile
            850             855             860

        Gln Asp Gln Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg
        865             870             875                 880
 20
        Arg Gln Glu Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile
                    885             890                 895

        Thr Gln Leu Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met
 25             900             905             910

        Ala Gln Lys Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln
                915             920             925

 30     Ala Ile Tyr Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr
            930             935             940

        Asn Trp Met Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp
        945             950             955             960
 35
        Ser Thr Leu Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu
                    965             970             975

        Leu Gly Glu Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn
 40             980             985             990

        Asp Leu Trp Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu
                995             1000            1005

 45     Gln Lys Leu Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu
            1010            1015            1020

        Ala Ile Arg Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu
        1025            1030            1035            1040
 50
        Ser Glu Asn Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser
                    1045            1050            1055

        Gly Gly Val Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu
 55             1060            1065            1070

        Asp Leu Ser Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu
                1075            1080            1085

 60     Lys Lys Arg Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu
            1090            1095            1100

        Gly Pro Tyr Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile
        1105            1110            1115            1120
 65
        Ala Ala Leu Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp
                    1125            1130            1135

 70     Phe Asn Asp Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr
                1140            1145            1150
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SUBSTITUTE SHEET (RULE 26)

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      Gly Thr Leu Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln
              1155                    1160                  1165

  5   His Glu Leu Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr
              1170                    1175              1180

      Ile Ile Arg Asp Ala  *
      1185                 1190

 10

      (2) INFORMATION FOR SEQ ID NO:27:

           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 1881 base pairs
 15            (B) TYPE: nucleic acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear
          (ii) MOLECULE TYPE: DNA (genomic)
          (ix) FEATURE:
 20            (A) NAME/KEY: CDS
               (B) LOCATION: 1..1881
               (D) OTHER INFORMATION: tcaB_i


          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27 (tcaB_i coding region):
 25
      ATG TCT GAA TCT TTA TTT ACA CAA ACG TTG AAA GAA GCG CGC CGT GAT    48
      Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
       1               5                  10                  15

 30   GCA TTG GTT GCT CAT TAT ATT GCT ACT CAG GTG CCC GCA GAT TTA AAA    96
      Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
                      20                  25                  30

      GAG AGT ATC CAG ACC GCG GAT GAT CTG TAC GAA TAT CTG TTG CTG GAT   144
 35   Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
                  35                  40                  45

      ACC AAA ATT AGC GAT CTG GTT ACT ACT TCA CCG CTG TCC GAA GCG ATT   192
      Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
 40       50                  55                  60

      GGC AGT CTG CAA TTG TTT ATT CAT CGT GCG ATA GAG GGC TAT GAC GGC   240
      Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
       65                  70                  75                  80
 45
      ACG CTG GCA GAC TCA GCA AAA CCC TAT TTT GCC GAT GAA CAG TTT TTA   288
      Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
                      85                  90                  95

 50   TAT AAC TGG GAT AGT TTT AAC CAC CGT TAT AGC ACT TGG GCT GGC AAG   336
      Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
                     100                 105                 110

      GAA CGG TTG AAA TTC TAT GCC GGG GAT TAT ATT GAT CCA ACA TTG CGA   384
 55   Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
                     115                 120                 125

      TTG AAT AAG ACC GAG ATA TTT ACC GCA TTT GAA CAA GGT ATT TCT CAA   432
      Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
 60       130                 135                 140

      GGG AAA TTA AAA AGT GAA TTA GTC GAA TCT AAA TTA CGT GAT TAT CTA   480
      Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
      145                 150                 155                 160
 65
      ATT AGT TAT GAC ACT TTA GCC ACC CTT GAT TAT ATT ACT GCC TGC CAA   528
      Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
                     165                 170                 175
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SUBSTITUTE SHEET (RULE 26)

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      GGC AAA GAT AAT AAA ACC ATC TTC TTT ATT GGC CGT ACA CAG AAT GCA  576
      Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
                  180             185                 190
  5
      CCC TAT GCA TTT TAT TGG CGA AAA TTA ACT TTA GTC ACT GAT GGC GGT  624
      Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
                  195             200                 205
 10
      AAG TTG AAA CCA GAT CAA TGG TCA GAG TGG CGA GCA ATT AAT GCC GGG  672
      Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
                  210             215                 220
 15
      ATT AGT GAG GCA TAT TCA GGG CAT GTC GAG CCT TTC TGG GAA AAT AAC  720
      Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
      225             230                 235                 240
 20
      AAG CTG CAC ATC CGT TGG TTT ACT ATC TCG AAA GAA GAT AAA ATA GAT  768
      Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Ile Asp
                  245                 250                 255
 20
      TTT GTT TAT AAA AAC ATC TGG GTG ATG AGT AGC GAT TAT AGC TGG GCA  816
      Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
                  260                 265                 270
 25
      TCA AAG AAA AAA ATC TTG GAA CTT TCT TTT ACT GAC TAC AAT AGA GTT  864
      Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
                  275                 280                 285
 30
      GGA GCA ACA GGA TCA TCA AGC CCG ACT GAA GTA GCT TCA CAA TAT GGT  912
      Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
                  290                 295                 300
 35
      TCT GAT GCT CAG ATG AAT ATT TCT GAT GAT GGG ACT GTA CTT ATT TTT  960
      Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
      305                 310                 315                 320
 40
      CAG AAT GCC GGC GGA GCT ACT CCC AGT ACT GGA GTG ACG TTA TGT TAT 1008
      Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
                  325                 330                 335
 40
      GAC TCT GGC AAC GTG ATT AAG AAC CTA TCT AGT ACA GGA AGT GCA AAT 1056
      Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
                  340                 345                 350
 45
      TTA TCG TCA AAG GAT TAT GCC ACA ACT AAA TTA CGC ATG TGT CAT GGA 1104
      Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
                  355                 360                 365
 50
      CAA AGT TAC AAT GAT AAT AAC TAC TGC AAT TTT ACA CTC TCT ATT AAT 1152
      Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
                  370                 375                 380
 55
      ACA ATA GAA TTC ACC TCC TAC GGC ACA TTC TCA TCA GAT GGA AAA CAA 1200
      Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
      385                 390                 395                 400
 60
      TTT ACA CCA CCT TCT GGT TCT GCC ATT GAT TTA CAC CTC CCT AAT TAT 1248
      Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
                  405                 410                 415
 60
      GTA GAT CTC AAC GCG CTA TTA GAT ATT AGC CTC GAT TCA CTA CTT AAT 1296
      Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn
                  420                 425                 430
 65
      TAT GAC GTT CAG GGG CAG TTT GGC GGA TCT AAT CCG GTT GAT AAT TTC 1344
      Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
                  435                 440                 445
 70
      AGT GGT CCC TAT GGT ATT TAT CTA TGG GAA ATC TTC TTC CAT ATT CCG 1392
      Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
```

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SUBSTITUTE SHEET (RULE 26)

```
              450                    455                    460
     TTC CTT GTT ACG GTC CGT ATG CAA ACC GAA CAA CGT TAC GAA GAC GCG 1440
     Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
 5   465                    470                    475                    480

     GAC ACT TGG TAC AAA TAT ATT TTC CGC AGC GCC GGT TAT CGC GAT GCT 1488
     Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                        485                    490                    495
10
     AAT GGC CAG CTC ATT ATG GAT GGC AGT AAA CCA CGT TAT TGG AAT GTG 1536
     Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
                        500                    505                    510

15   ATG CCA TTG CAA CTG GAT ACC GCA TGG GAT ACC ACA CAG CCC GCC ACC 1584
     Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
                        515                    520                    525

     ACT GAT CCA GAT GTG ATC GCT ATG GCG GAC CCG ATG CAT TAC AAG CTG 1632
     Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
20                       530                    535                    540

     GCG ATA TTC CTG CAT ACC CTT GAT CTA TTG ATT GCC CGA GGC GAC AGC 1680
     Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
25   545                    550                    555                    560

     GCT TAC CGT CAA CTT GAA CGC GAT ACT CTA GTC GAA GCC AAA ATG TAC 1728
     Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
                        565                    570                    575
30
     TAC ATT CAG GCA CAA CAG CTA CTG GGA CCG CGC CCT GAT ATC CAT ACC 1776
     Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
                        580                    585                    590

35   ACC AAT ACT TGG CCA AAT CCC ACC TTG AGT AAA GAA GCT GGC GCT ATT 1824
     Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
                        595                    600                    605

     GCC ACA CCG ACA TTC CTC AGT TCA CCG GAG GTG ATG ACG TTC GCT GCC 1872
40   Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
     610                    615                    620

     TGG CTA AGC                                                       1881
     Trp Leu Ser
45   625
```


(2) INFORMATION FOR SEQ ID NO:28:

50   (i) SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 627 amino acids
                 (B) TYPE: amino acid
                 (D) TOPOLOGY: linear
           (ii) MOLECULE TYPE: protein
55
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28 (TcaB$_i$ protein):

```
     Met Ser Glu Ser Leu Phe Thr Gln Thr Leu Lys Glu Ala Arg Arg Asp
     1               5                   10                  15
60
     Ala Leu Val Ala His Tyr Ile Ala Thr Gln Val Pro Ala Asp Leu Lys
                     20                  25                  30

     Glu Ser Ile Gln Thr Ala Asp Asp Leu Tyr Glu Tyr Leu Leu Leu Asp
65                   35                  40                  45

     Thr Lys Ile Ser Asp Leu Val Thr Thr Ser Pro Leu Ser Glu Ala Ile
                     50                  55                  60
```

SUBSTITUTE SHEET (RULE 26)

```
      Gly Ser Leu Gln Leu Phe Ile His Arg Ala Ile Glu Gly Tyr Asp Gly
      65                  70                  75                  80

  5   Thr Leu Ala Asp Ser Ala Lys Pro Tyr Phe Ala Asp Glu Gln Phe Leu
                      85                  90                  95

      Tyr Asn Trp Asp Ser Phe Asn His Arg Tyr Ser Thr Trp Ala Gly Lys
                  100                 105                 110

 10   Glu Arg Leu Lys Phe Tyr Ala Gly Asp Tyr Ile Asp Pro Thr Leu Arg
                  115                 120                 125

      Leu Asn Lys Thr Glu Ile Phe Thr Ala Phe Glu Gln Gly Ile Ser Gln
                  130                 135                 140

 15   Gly Lys Leu Lys Ser Glu Leu Val Glu Ser Lys Leu Arg Asp Tyr Leu
      145                 150                 155                 160

      Ile Ser Tyr Asp Thr Leu Ala Thr Leu Asp Tyr Ile Thr Ala Cys Gln
 20                   165                 170                 175

      Gly Lys Asp Asn Lys Thr Ile Phe Phe Ile Gly Arg Thr Gln Asn Ala
                  180                 185                 190

 25   Pro Tyr Ala Phe Tyr Trp Arg Lys Leu Thr Leu Val Thr Asp Gly Gly
                  195                 200                 205

      Lys Leu Lys Pro Asp Gln Trp Ser Glu Trp Arg Ala Ile Asn Ala Gly
          210                 215                 220

 30   Ile Ser Glu Ala Tyr Ser Gly His Val Glu Pro Phe Trp Glu Asn Asn
      225                 230                 235                 240

      Lys Leu His Ile Arg Trp Phe Thr Ile Ser Lys Glu Asp Lys Ile Asp
 35                   245                 250                 255

      Phe Val Tyr Lys Asn Ile Trp Val Met Ser Ser Asp Tyr Ser Trp Ala
                  260                 265                 270

 40   Ser Lys Lys Lys Ile Leu Glu Leu Ser Phe Thr Asp Tyr Asn Arg Val
          275                 280                 285

      Gly Ala Thr Gly Ser Ser Ser Pro Thr Glu Val Ala Ser Gln Tyr Gly
          290                 295                 300

 45   Ser Asp Ala Gln Met Asn Ile Ser Asp Asp Gly Thr Val Leu Ile Phe
      305                 310                 315                 320

 50   Gln Asn Ala Gly Gly Ala Thr Pro Ser Thr Gly Val Thr Leu Cys Tyr
                  325                 330                 335

      Asp Ser Gly Asn Val Ile Lys Asn Leu Ser Ser Thr Gly Ser Ala Asn
                  340                 345                 350

 55   Leu Ser Ser Lys Asp Tyr Ala Thr Thr Lys Leu Arg Met Cys His Gly
                  355                 360                 365

      Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu Ser Ile Asn
          370                 375                 380

 60   Thr Ile Glu Phe Thr Ser Tyr Gly Thr Phe Ser Ser Asp Gly Lys Gln
      385                 390                 395                 400

 65   Phe Thr Pro Pro Ser Gly Ser Ala Ile Asp Leu His Leu Pro Asn Tyr
                  405                 410                 415

      Val Asp Leu Asn Ala Leu Leu Asp Ile Ser Leu Asp Ser Leu Leu Asn
                  420                 425                 430

 70   Tyr Asp Val Gln Gly Gln Phe Gly Gly Ser Asn Pro Val Asp Asn Phe
                  435                 440                 445
```

SUBSTITUTE SHEET (RULE 26)

```
        Ser Gly Pro Tyr Gly Ile Tyr Leu Trp Glu Ile Phe Phe His Ile Pro
            450             455             460
   5    Phe Leu Val Thr Val Arg Met Gln Thr Glu Gln Arg Tyr Glu Asp Ala
        465             470             475             480

        Asp Thr Trp Tyr Lys Tyr Ile Phe Arg Ser Ala Gly Tyr Arg Asp Ala
                    485             490             495
  10
        Asn Gly Gln Leu Ile Met Asp Gly Ser Lys Pro Arg Tyr Trp Asn Val
                    500             505             510

        Met Pro Leu Gln Leu Asp Thr Ala Trp Asp Thr Thr Gln Pro Ala Thr
  15            515             520             525

        Thr Asp Pro Asp Val Ile Ala Met Ala Asp Pro Met His Tyr Lys Leu
            530             535             540
  20    Ala Ile Phe Leu His Thr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ser
        545             550             555             560

        Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Val Glu Ala Lys Met Tyr
                    565             570             575
  25
        Tyr Ile Gln Ala Gln Gln Leu Leu Gly Pro Arg Pro Asp Ile His Thr
                    580             585             590

        Thr Asn Thr Trp Pro Asn Pro Thr Leu Ser Lys Glu Ala Gly Ala Ile
  30            595             600             605

        Ala Thr Pro Thr Phe Leu Ser Ser Pro Glu Val Met Thr Phe Ala Ala
            610             615             620
  35    Trp Leu Ser
        625



  40    (2)  INFORMATION FOR SEQ ID NO:29:

               (i)  SEQUENCE CHARACTERISTICS:
                    (A)  LENGTH: 1689 base pairs
                    (B)  TYPE: nucleic acid
                    (C)  STRANDEDNESS: double
  45                (D)  TOPOLOGY: linear
              (ii)  MOLECULE TYPE: DNA (genomic)
              (ix)  FEATURE:
                    (A)  NAME/KEY: CDS
                    (B)  LOCATION: 1..1689
  50                (D)  OTHER INFORMATION: tcaB_{ii}


         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:29 (tcaB_{ii} coding regaion):

        GCA GGC GAT ACC GCA AAT ATT GGC GAC GGT GAT TTC TTG CCA CCG TAC   48
  55    Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr
         1               5               10              15

        AAC GAT GTA CTA CTC GGT TAC TGG GAT AAA CTT GAG TTA CGC CTA TAC   96
        Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu Arg Leu Tyr
  60                20              25              30

        AAC CTG CGC CAC AAT CTG AGT CTG GAT GGT CAA CCG CTA AAT CTG CCA  144
        Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu Asn Leu Pro
                    35              40              45
  65
        CTG TAT GCC ACG CCG GTA GAC CCG AAA ACC CTG CAA CGC CAG CAA GCC  192
        Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala
                    50              55              60
```

SUBSTITUTE SHEET (RULE 26)

GGA GGG GAC GGT ACA GGC AGT AGT CCG GCT GGT GGT CAA GGC AGT GTT 240
Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln Gly Ser Val
 65   70   75   80

CAG GGC TGG CGC TAT CCG TTA TTG GTA GAA CGC GCC CGC TCT GCC GTG 288
Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg Ser Ala Val
                85   90   95

AGT TTG TTG ACT CAG TTC GGC AAC AGC TTA CAA ACA ACG TTA GAA CAT 336
Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr Leu Glu His
               100   105   110

CAG GAT AAT GAA AAA ATG ACG ATA CTG TTG CAG ACT CAA CAG GAA GCC 384
Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln Gln Glu Ala
           115   120   125

ATC CTG AAA CAT CAG CAC GAT ATA CAA CAA AAT AAT CTA AAA GGA TTA 432
Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu Lys Gly Leu
       130   135   140

CAA CAC AGC CTG ACC GCA TTA CAG GCT AGC CGT GAT GGC GAC ACA TTG 480
Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly Asp Thr Leu
145   150   155   160

CGG CAA AAA CAT TAC AGC GAC CTG ATT AAC GGT GGT CTA TCT GCG GCA 528
Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu Ser Ala Ala
                165   170   175

GAA ATC GCC GGT CTG ACA CTA CGC AGC ACC GCC ATG ATT ACC AAT GGC 576
Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile Thr Asn Gly
                180   185   190

GTT GCA ACG GGA TTG CTG ATT GCC GGC GGA ATC GCC AAC GCG GTA CCT 624
Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn Ala Val Pro
           195   200   205

AAC GTC TTC GGG CTG GCT AAC GGT GGA TCG GAA TGG GGA GCG CCA TTA 672
Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly Ala Pro Leu
       210   215   220

ATT GGC TCC GGG CAA GCA ACC CAA GTT GGC GCC GGC ATC CAG GAT CAG 720
Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile Gln Asp Gln
225   230   235   240

AGC GCG GGC ATT TCA GAA GTG ACA GCA GGC TAT CAG CGT CGT CAG GAA 768
Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg Arg Gln Glu
                245   250   255

GAA TGG GCA TTG CAA CGG GAT ATT GCT GAT AAC GAA ATA ACC CAA CTG 816
Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile Thr Gln Leu
           260   265   270

GAT GCC CAG ATA CAA AGC CTG CAA GAG CAA ATC ACG ATG GCA CAA AAA 864
Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met Ala Gln Lys
           275   280   285

CAG ATC ACG CTC TCT GAA ACC GAA CAA GCG AAT GCC CAA GCG ATT TAT 912
Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln Ala Ile Tyr
       290   295   300

GAC CTG CAA ACC ACT CGT TTT ACC GGG CAG GCA CTG TAT AAC TGG ATG 960
Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr Asn Trp Met
305   310   315   320

GCC GGT CGT CTC TCC GCG CTC TAT TAC CAA ATG TAT GAT TCC ACT CTG 1008
Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp Ser Thr Leu
                325   330   335

CCA ATC TGT CTC CAG CCA AAA GCC GCA TTA GTA CAG GAA TTA GGC GAG 1056
Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu Leu Gly Glu

SUBSTITUTE SHEET (RULE 26)

```
                     340                    345        .            350
        AAA GAG AGC GAC AGT CTT TTC CAG GTT CCG GTG TGG AAT GAT CTG TGG 1104
        Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn Asp Leu Trp
 5             355                    360                365

        CAA GGG CTG TTA GCA GGA GAA GGT TTA AGT TCA GAG CTA CAG AAA CTG 1152
        Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu Gln Lys Leu
               370                    375                380
10
        GAT GCC ATC TGG CTT GCA CGT GGT GGT ATT GGG CTA GAA GCC ATC CGC 1200
        Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu Ala Ile Arg
        385                    390                395                400

15      ACC GTG TCG CTG GAT ACC CTG TTT GGC ACA GGG ACG TTA AGT GAA AAT 1248
        Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu Ser Glu Asn
                       405                    410                415

        ATC AAT AAA GTG CTT AAC GGG GAA ACG GTA TCT CCA TCC GGT GGC GTC 1296
20      Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser Gly Gly Val
                   420                    425                430

        ACT CTG GCG CTG ACA GGG GAT ATC TTC CAA GCA ACA CTG GAT TTG AGT 1344
        Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu Asp Leu Ser
25             435                    440                445

        CAG CTA GGT TTG GAT AAC TCT TAC AAC TTG GGT AAC GAG AAG AAA CGT 1392
        Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu Lys Lys Arg
               450                    455                460
30
        CGT ATT AAA CGT ATC GCC GTC ACC CTG CCA ACA CTT CTG GGG CCA TAT 1440
        Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu Gly Pro Tyr
        465                    470                475                480

35      CAA GAT CTT GAA GCC ACA CTG GTA ATG GGT GCG GAA ATC GCC GCC TTA 1488
        Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile Ala Ala Leu
                       485                    490                495

        TCA CAC GGT GTG AAT GAC GGA GGC CGG TTT GTT ACC GAC TTT AAC GAC 1536
40      Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp Phe Asn Asp
                   500                    505                510

        AGC CGT TTT CTG CCT TTT GAA GGT CGA GAT GCA ACA ACC GGC ACA CTG 1584
        Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr Gly Thr Leu
45             515                    520                525

        GAG CTC AAT ATT TTC CAT GCG GGT AAA GAG GGA ACG CAA CAC GAG TTG 1632
        Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln His Glu Leu
               530                    535                540
50
        GTC GCG AAT CTG AGT GAC ATC ATT GTG CAT CTG AAT TAC ATC ATT CGA 1680
        Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr Ile Ile Arg
        545                    550                555                560

55      GAC GCG TAA                                                      1689
        Asp Ala  *
```

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 562 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein

SUBSTITUTE SHEET (RULE 26)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30 (TcaB$_{ii}$ protein):

```
        Ala Gly Asp Thr Ala Asn Ile Gly Asp Gly Asp Phe Leu Pro Pro Tyr
         1               5               10              15
  5
        Asn Asp Val Leu Leu Gly Tyr Trp Asp Lys Leu Glu Leu Arg Leu Tyr
                     20              25              30
        Asn Leu Arg His Asn Leu Ser Leu Asp Gly Gln Pro Leu Asn Leu Pro
 10              35              40              45
        Leu Tyr Ala Thr Pro Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala
             50              55              60
        Gly Gly Asp Gly Thr Gly Ser Ser Pro Ala Gly Gly Gln Gly Ser Val
 15      65              70              75               80
        Gln Gly Trp Arg Tyr Pro Leu Leu Val Glu Arg Ala Arg Ser Ala Val
                     85              90              95
 20
        Ser Leu Leu Thr Gln Phe Gly Asn Ser Leu Gln Thr Thr Leu Glu His
                    100             105             110
        Gln Asp Asn Glu Lys Met Thr Ile Leu Leu Gln Thr Gln Gln Glu Ala
 25             115             120             125
        Ile Leu Lys His Gln His Asp Ile Gln Gln Asn Asn Leu Lys Gly Leu
            130             135             140
 30     Gln His Ser Leu Thr Ala Leu Gln Ala Ser Arg Asp Gly Asp Thr Leu
        145             150             155             160
        Arg Gln Lys His Tyr Ser Asp Leu Ile Asn Gly Gly Leu Ser Ala Ala
                    165             170             175
 35
        Glu Ile Ala Gly Leu Thr Leu Arg Ser Thr Ala Met Ile Thr Asn Gly
                    180             185             190
        Val Ala Thr Gly Leu Leu Ile Ala Gly Gly Ile Ala Asn Ala Val Pro
 40         195             200             205
        Asn Val Phe Gly Leu Ala Asn Gly Gly Ser Glu Trp Gly Ala Pro Leu
            210             215             220
 45     Ile Gly Ser Gly Gln Ala Thr Gln Val Gly Ala Gly Ile Gln Asp Gln
        225             230             235             240
        Ser Ala Gly Ile Ser Glu Val Thr Ala Gly Tyr Gln Arg Arg Gln Glu
                    245             250             255
 50
        Glu Trp Ala Leu Gln Arg Asp Ile Ala Asp Asn Glu Ile Thr Gln Leu
                    260             265             270
        Asp Ala Gln Ile Gln Ser Leu Gln Glu Gln Ile Thr Met Ala Gln Lys
 55         275             280             285
        Gln Ile Thr Leu Ser Glu Thr Glu Gln Ala Asn Ala Gln Ala Ile Tyr
            290             295             300
 60     Asp Leu Gln Thr Thr Arg Phe Thr Gly Gln Ala Leu Tyr Asn Trp Met
        305             310             315             320
        Ala Gly Arg Leu Ser Ala Leu Tyr Tyr Gln Met Tyr Asp Ser Thr Leu
                    325             330             335
 65
        Pro Ile Cys Leu Gln Pro Lys Ala Ala Leu Val Gln Glu Leu Gly Glu
                    340             345             350
        Lys Glu Ser Asp Ser Leu Phe Gln Val Pro Val Trp Asn Asp Leu Trp
 70         355             360             365
```

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```
     Gln Gly Leu Leu Ala Gly Glu Gly Leu Ser Ser Glu Leu Gln Lys Leu
         370             375             380
 5   Asp Ala Ile Trp Leu Ala Arg Gly Gly Ile Gly Leu Glu Ala Ile Arg
     385             390             - 395             400

     Thr Val Ser Leu Asp Thr Leu Phe Gly Thr Gly Thr Leu Ser Glu Asn
                 405             410             415
10
     Ile Asn Lys Val Leu Asn Gly Glu Thr Val Ser Pro Ser Gly Gly Val
                 420             425             430

     Thr Leu Ala Leu Thr Gly Asp Ile Phe Gln Ala Thr Leu Asp Leu Ser
15           435             440             445

     Gln Leu Gly Leu Asp Asn Ser Tyr Asn Leu Gly Asn Glu Lys Lys Arg
         450             455             460

20   Arg Ile Lys Arg Ile Ala Val Thr Leu Pro Thr Leu Leu Gly Pro Tyr
     465             470             475             480

     Gln Asp Leu Glu Ala Thr Leu Val Met Gly Ala Glu Ile Ala Ala Leu
                 485             490             495
25
     Ser His Gly Val Asn Asp Gly Gly Arg Phe Val Thr Asp Phe Asn Asp
                 500             505             510

     Ser Arg Phe Leu Pro Phe Glu Gly Arg Asp Ala Thr Thr Gly Thr Leu
30       515             520             525

     Glu Leu Asn Ile Phe His Ala Gly Lys Glu Gly Thr Gln His Glu Leu
         530             535             540

35   Val Ala Asn Leu Ser Asp Ile Ile Val His Leu Asn Tyr Ile Ile Arg
     545             550             555             560

     Asp Ala  *

40

     (2)  INFORMATION FOR SEQ ID NO:31:

          (i)  SEQUENCE CHARACTERISTICS:
45             (A)  LENGTH: 4458 base pairs
               (B)  TYPE: nucleic acid
               (C)  STRANDEDNESS: double
               (D)  TOPOLOGY: linear
          (ii) MOLECULE TYPE: DNA (genomic)
50        (ix) FEATURE:
               (A)  NAME/KEY: CDS
               (B)  LOCATION: 1..4458

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31 (tcac gene):
55
     ATG CAG GAT TCA CCA GAA GTA TCG ATT ACA ACG CTG TCA CTT CCC AAA      48
     Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys
       1           5               10              15

60   GGT GGC GGT GCT ATC AAT GGC ATG GGA GAA GCA CTG AAT GCT GCC GGC      96
     Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly
                 20              25              30

     CCT GAT GGA ATG GCC TCC CTA TCT CTG CCA TTA CCC CTT TCG ACC GGC     144
65   Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly
                 35              40              45

     AGA GGG ACG GCT CCT GGA TTA TCG CTG ATT TAC AGC AAC AGT GCA GGT     192
     Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly
```

SUBSTITUTE SHEET (RULE 26)

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            50                      55              .        60
      AAT GGG CCT TTC GGC ATC GGC TGG CAA TGC GGT GTT ATG TCC ATT AGC  240
      Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser
  5   65                  70              75                  80

      CGA CGC ACC CAA CAT GGC ATT CCA CAA TAC GGT AAT GAC GAC ACG TTC  288
      Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe
                          85                  90                  95
 10
      CTA TCC CCA CAA GGC GAG GTC ATG AAT ATC GCC CTG AAT GAC CAA GGG  336
      Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly
                      100             105             110

 15   CAA CCT GAT ATC CGT CAA GAC GTT AAA ACG CTG CAA GGC GTT ACC TTG  384
      Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu
                      115             120             125

      CCA ATT TCC TAT ACC GTG ACC CGC TAT CAA GCC CGC CAG ATC CTG GAT  432
 20   Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp
                  130             135             140

      TTC AGT AAA ATC GAA TAC TGG CAA CCT GCC TCC GGT CAA GAA GGA CGC  480
      Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg
 25   145             150             155             160

      GCT TTC TGG CTG ATA TCG ACA CCG GAC GGG CAT CTA CAC ATC TTA GGG  528
      Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly
                      165             170             175
 30
      AAA ACC GCG CAG GCT TGT CTG GCA AAT CCG CAA AAT GAC CAA CAA ATC  576
      Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile
                      180             185             190

 35   GCC CAG TGG TTG CTG GAA GAA ACT GTG ACG CCA GCC GGT GAA CAT GTC  624
      Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val
                  195             200             205

      AGC TAT CAA TAT CGA GCC GAA GAT GAA GCC CAT TGT GAC GAC AAT GAA  672
 40   Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu
                  210             215             220

      AAA ACC GCT CAT CCC AAT GTT ACC GCA CAG CGC TAT CTG GTA CAG GTG  720
      Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val
 45   225             230             235             240

      AAC TAC GGC AAC ATC AAA CCA CAA GCC AGC CTG TTC GTA CTG GAT AAC  768
      Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn
                      245             250             255
 50
      GCA CCT CCC GCA CCG GAA GAG TGG CTG TTT CAT CTG GTC TTT GAC CAC  816
      Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His
                      260             265             270

 55   GGT GAG CGC GAT ACC TCA CTT CAT ACC GTG CCA ACA TGG GAT GCA GGT  864
      Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly
                  275             280             285

      ACA GCG CAA TGG TCT GTA CGC CCG GAT ATC TTC TCT CGC TAT GAA TAT  912
 60   Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr
                  290             295             300

      GGT TTT GAA GTG CGT ACT CGC CGC TTA TGT CAA CAA GTG CTG ATG TTT  960
      Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe
 65   305             310             315             320

      CAC CGC ACC GCG CTC ATG GCC GGA GAA GCC AGT ACC AAT GAC GCC CCG 1008
      His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro
                      325             330             335
 70
      GAA CTG GTT GGA CGC TTA ATA CTG GAA TAT GAC AAA AAC GCC AGC GTC 1056
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SUBSTITUTE SHEET (RULE 26)

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           Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val
                    340           345               350

     5     ACC ACG TTG ATT ACC ATC CGT CAA TTA AGC CAT GAA TCG GAC GGG AGG 1104
           Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg
                    355               360               365

           CCA GTC ACC CAG CCA CCA CTA GAA CTA GCC TGG CAA CGG TTT GAT CTG 1152
    10     Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu
                    370               375               380

           GAG AAA ATC CCG ACA TGG CAA CGC TTT GAC GCA CTA GAT AAT TTT AAC 1200
           Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn
           385               390               395               400
    15
           TCG CAG CAA CGT TAT CAA CTG GTT GAT CTG CGG GGA GAA GGG TTG CCA 1248
           Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro
                              405               410               415

    20     GGT ATG CTG TAT CAA GAT CGA GGC GCT TGG TGG TAT AAA GCT CCG CAA 1296
           Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln
                              420               425               430

    25     CGT CAG GAA GAC GGA GAC AGC AAT GCC GTC ACT TAC GAC AAA ATC GCC 1344
           Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala
                    435               440               445

           CCA CTG CCT ACC CTA CCC AAT TTG CAG GAT AAT GCC TCA TTG ATG GAT 1392
    30     Pro Leu Pro Thr Leu Pro Asn Leu Gln Asp Asn Ala Ser Leu Met Asp
                    450               455               460

           ATC AAC GGA GAC GGC CAA CTG GAT TGG GTT GTT ACC GCC TCC GGT ATT 1440
           Ile Asn Gly Asp Gly Gln Leu Asp Trp Val Val Thr Ala Ser Gly Ile
           465               470               475               480
    35
           CGC GGA TAC CAT AGT CAG CAA CCC GAT GGA AAG TGG ACG CAC TTT ACG 1488
           Arg Gly Tyr His Ser Gln Gln Pro Asp Gly Lys Trp Thr His Phe Thr
                              485               490               495

    40     CCA ATC AAT GCC TTG CCC GTG GAA TAT TTT CAT CCA AGC ATC CAG TTC 1536
           Pro Ile Asn Ala Leu Pro Val Glu Tyr Phe His Pro Ser Ile Gln Phe
                    500               505               510

    45     GCT GAC CTT ACC GGG GCA GGC TTA TCT GAT TTA GTG TTG ATC GGG CCG 1584
           Ala Asp Leu Thr Gly Ala Gly Leu Ser Asp Leu Val Leu Ile Gly Pro
                    515               520               525

           AAA AGC GTG CGT CTA TAT GCC AAC CAG CGA AAC GGC TGG CGT AAA GGA 1632
           Lys Ser Val Arg Leu Tyr Ala Asn Gln Arg Asn Gly Trp Arg Lys Gly
    50              530               535               540

           GAA GAT GTC CCC CAA TCC ACA GGT ATC ACC CTG CCT GTC ACA GGG ACC 1680
           Glu Asp Val Pro Gln Ser Thr Gly Ile Thr Leu Pro Val Thr Gly Thr
           545               550               555               560
    55
           GAT GCC CGC AAA CTG GTG GCT TTC AGT GAT ATG CTC GGT TCC GGT CAA 1728
           Asp Ala Arg Lys Leu Val Ala Phe Ser Asp Met Leu Gly Ser Gly Gln
                    565               570               575

    60     CAA CAT CTG GTG GAA ATC AAG GGT AAT CGC GTC ACC TGT TGG CCG AAT 1776
           Gln His Leu Val Glu Ile Lys Gly Asn Arg Val Thr Cys Trp Pro Asn
                    580               585               590

    65     CTA GGG CAT GGC CGT TTC GGT CAA CCA CTA ACT CTG TCA GGA TTT AGC 1824
           Leu Gly His Gly Arg Phe Gly Gln Pro Leu Thr Leu Ser Gly Phe Ser
                    595               600               605

           CAG CCC GAA AAT AGC TTC AAT CCC GAA CGG CTG TTT CTG GCG GAT ATC 1872
    70     Gln Pro Glu Asn Ser Phe Asn Pro Glu Arg Leu Phe Leu Ala Asp Ile
                    610               615               620
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SUBSTITUTE SHEET (RULE 26)

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      GAC GGC TCC GGC ACC ACC GAC CTT ATC TAT GCG CAA TCC GGC TCT TTG 1920
      Asp Gly Ser Gly Thr Thr Asp Leu Ile Tyr Ala Gln Ser Gly Ser Leu
      625                 630                 635                 640

 5    CTC ATT TAT CTC AAC CAA AGT GGT AAT CAG TTT GAT GCC CCG TTG ACA 1968
      Leu Ile Tyr Leu Asn Gln Ser Gly Asn Gln Phe Asp Ala Pro Leu Thr
                          645                 650                 655

      TTA GCG TTG CCA GAA GGC GTA CAA TTT GAC AAC ACT TGC CAA CTT CAA 2016
10    Leu Ala Leu Pro Glu Gly Val Gln Phe Asp Asn Thr Cys Gln Leu Gln
                      660                 665                 670

      GTC GCC GAT ATT CAG GGA TTA GGG ATA GCC AGC TTG ATT CTG ACT GTG 2064
15    Val Ala Asp Ile Gln Gly Leu Gly Ile Ala Ser Leu Ile Leu Thr Val
                  675                 680                 685

      CCA CAT ATC GCG CCA CAT CAC TGG CGT TGT GAC CTG TCA CTG ACC AAA 2112
      Pro His Ile Ala Pro His His Trp Arg Cys Asp Leu Ser Leu Thr Lys
              690                 695                 700
20
      CCC TGG TTG TTG AAT GTA ATG AAC AAT AAC CGG GGC GCA CAT CAC ACG 2160
      Pro Trp Leu Leu Asn Val Met Asn Asn Asn Arg Gly Ala His His Thr
      705                 710                 715                 720

25    CTA CAT TAT CGT AGT TCC GCG CAA TTC TGG TTG GAT GAA AAA TTA CAG 2208
      Leu His Tyr Arg Ser Ser Ala Gln Phe Trp Leu Asp Glu Lys Leu Gln
                          725                 730                 735

      CTC ACC AAA GCA GGC AAA TCT CCG GCT TGT TAT CTG CCG TTT CCA ATG 2256
30    Leu Thr Lys Ala Gly Lys Ser Pro Ala Cys Tyr Leu Pro Phe Pro Met
                      740                 745                 750

      CAT TTG CTA TGG TAT ACC GAA ATT CAG GAT GAA ATC AGC GGC AAC CGG 2304
      His Leu Leu Trp Tyr Thr Glu Ile Gln Asp Glu Ile Ser Gly Asn Arg
35                    755                 760                 765

      CTC ACC AGT GAA GTC AAC TAC AGC CAC GGC GTC TGG GAT GGT AAA GAG 2352
      Leu Thr Ser Glu Val Asn Tyr Ser His Gly Val Trp Asp Gly Lys Glu
                  770                 775                 780
40
      CGG GAA TTC AGA GGA TTT GGC TGC ATC AAA CAG ACA GAT ACC ACA ACG 2400
      Arg Glu Phe Arg Gly Phe Gly Cys Ile Lys Gln Thr Asp Thr Thr Thr
      785                 790                 795                 800

45    TTT TCT CAC GGC ACC GCC CCC GAA CAG GCG GCA CCG TCG CTG AGT ATT 2448
      Phe Ser His Gly Thr Ala Pro Glu Gln Ala Ala Pro Ser Leu Ser Ile
                          805                 810                 815

      AGC TGG TTT GCC ACC GGC ATG GAT GAA GTA GAC AGC CAA TTA GCT ACG 2496
50    Ser Trp Phe Ala Thr Gly Met Asp Glu Val Asp Ser Gln Leu Ala Thr
                      820                 825                 830

      GAA TAT TGG CAG GCA GAC ACG CAA GCT TAT AGC GGA TTT GAA ACC CGT 2544
      Glu Tyr Trp Gln Ala Asp Thr Gln Ala Tyr Ser Gly Phe Glu Thr Arg
55                    835                 840                 845

      TAT ACC GTC TGG GAT CAC ACC AAC CAG ACA GAC CAA GCA TTT ACC CCC 2592
      Tyr Thr Val Trp Asp His Thr Asn Gln Thr Asp Gln Ala Phe Thr Pro
                  850                 855                 860
60
      AAT GAG ACA CAA CGT AAC TGG CTG ACG CGA GCG CTT AAA GGC CAA CTG 2640
      Asn Glu Thr Gln Arg Asn Trp Leu Thr Arg Ala Leu Lys Gly Gln Leu
      865                 870                 875                 880

65    CTA CGC ACT GAG CTC TAC GGT CTG GAC GGA ACA GAT AAG CAA ACA GTG 2688
      Leu Arg Thr Glu Leu Tyr Gly Leu Asp Gly Thr Asp Lys Gln Thr Val
                          885                 890                 895

      CCT TAT ACC GTC AGT GAA TCG CGC TAT CAG GTA CGC TCT ATT CCC GTA 2736
70    Pro Tyr Thr Val Ser Glu Ser Arg Tyr Gln Val Arg Ser Ile Pro Val
                      900                 905                 910
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      AAT AAA GAA ACT GAA TTA TCT GCC TGG GTG ACT GCT ATT GAA AAT CGC 2784
      Asn Lys Glu Thr Glu Leu Ser Ala Trp Val Thr Ala Ile Glu Asn Arg
          915             920             925
  5

      AGC TAC CAC TAT GAA CGT ATC ATC ACT GAC CCA CAG TTC AGC CAG AGT 2832
      Ser Tyr His Tyr Glu Arg Ile Ile Thr Asp Pro Gln Phe Ser Gln Ser
          930             935             940

 10   ATC AAG TTG CAA CAC GAT ATC TTT GGT CAA TCA CTG CAA AGT GTC GAT 2880
      Ile Lys Leu Gln His Asp Ile Phe Gly Gln Ser Leu Gln Ser Val Asp
      945             950             955             960

      ATT GCC TGG CCG CGC CGC GAA AAA CCA GCA GTG AAT CCC TAC CCG CCT 2928
 15   Ile Ala Trp Pro Arg Arg Glu Lys Pro Ala Val Asn Pro Tyr Pro Pro
                  965             970             975

      ACC CTG CCG GAA ACG CTA TTT GAC AGC AGC TAT GAT GAT CAA CAA CAA 2976
      Thr Leu Pro Glu Thr Leu Phe Asp Ser Ser Tyr Asp Asp Gln Gln Gln
 20                   980             985             990

      CTA TTA CGT CTG GTG AGA CAA AAA AAT AGC TGG CAT CAC CTG ACT GAT 3024
      Leu Leu Arg Leu Val Arg Gln Lys Asn Ser Trp His His Leu Thr Asp
                  995             1000            1005
 25

      GGG GAA AAC TGG CGA TTA GGT TTA CCG AAT GCA CAA CGC CGT GAT GTT 3072
      Gly Glu Asn Trp Arg Leu Gly Leu Pro Asn Ala Gln Arg Arg Asp Val
          1010            1015            1020

 30   TAT ACT TAT GAC CGG AGC AAA ATT CCA ACC GAA GGG ATT TCC CTT GAA 3120
      Tyr Thr Tyr Asp Arg Ser Lys Ile Pro Thr Glu Gly Ile Ser Leu Glu
      1025            1030            1035            1040

      ATC TTG CTG AAA GAT GAT GGC CTG CTA GCA GAT GAA AAA GCG GCC GTT 3168
 35   Ile Leu Leu Lys Asp Asp Gly Leu Leu Ala Asp Glu Lys Ala Ala Val
                  1045            1050            1055

      TAT CTG GGA CAA CAA CAG ACG TTT TAC ACC GCC GGT CAA GCG GAA GTC 3216
      Tyr Leu Gly Gln Gln Gln Thr Phe Tyr Thr Ala Gly Gln Ala Glu Val
 40               1060            1065            1070

      ACT CTA GAA AAA CCC ACG TTA CAA GCA CTG GTC GCG TTC CAA GAA ACC 3264
      Thr Leu Glu Lys Pro Thr Leu Gln Ala Leu Val Ala Phe Gln Glu Thr
                  1075            1080            1085
 45

      GCC ATG ATG GAC GAT ACC TCA TTA CAG GCG TAT GAA GGC GTG ATT GAA 3312
      Ala Met Met Asp Asp Thr Ser Leu Gln Ala Tyr Glu Gly Val Ile Glu
          1090            1095            1100

 50   GAG CAA GAG TTG AAT ACC GCG CTG ACA CAG GCC GGT TAT CAG CAA GTC 3360
      Glu Gln Glu Leu Asn Thr Ala Leu Thr Gln Ala Gly Tyr Gln Gln Val
      1105            1110            1115            1120

      GCG CGG TTG TTT AAT ACC AGA TCA GAA AGC CCG GTA TGG GCG GCA CGG 3408
 55   Ala Arg Leu Phe Asn Thr Arg Ser Glu Ser Pro Val Trp Ala Ala Arg
                  1125            1130            1135

      CAA GGT TAT ACC GAT TAC GGT GAC GCC GCA CAG TTC TGG CGG CCT CAG 3456
      Gln Gly Tyr Thr Asp Tyr Gly Asp Ala Ala Gln Phe Trp Arg Pro Gln
 60               1140            1145            1150

      GCT CAG CGT AAC TCG TTG CTG ACA GGG AAA ACC ACA CTG ACC TGG GAT 3504
      Ala Gln Arg Asn Ser Leu Leu Thr Gly Lys Thr Thr Leu Thr Trp Asp
                  1155            1160            1165
 65

      ACC CAT CAT TGT GTA ATA ATA CAG ACT CAA GAT GCC GCT GGA TTA ACG 3552
      Thr His His Cys Val Ile Ile Gln Thr Gln Asp Ala Ala Gly Leu Thr
          1170            1175            1180

 70   ACG CAA GCC CAT TAC GAT TAT CGT TTC CTT ACA CCG GTA CAA CTG ACA 3600
      Thr Gln Ala His Tyr Asp Tyr Arg Phe Leu Thr Pro Val Gln Leu Thr
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SUBSTITUTE SHEET (RULE 26)

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        1185                    1190              . 1195                    1200
        GAT ATT AAT GAT AAT CAA CAT ATT GTG ACT CTG GAC GCG CTA GGT CGC 3648
  5     Asp Ile Asn Asp Asn Gln His Ile Val Thr Leu Asp Ala Leu Gly Arg
                            1205              1210                    1215

        GTA ACC ACC AGC CGG TTC TGG GGC ACA GAG GCA GGA CAA GCC GCA GGC 3696
        Val Thr Thr Ser Arg Phe Trp Gly Thr Glu Ala Gly Gln Ala Ala Gly
 10                     1220              1225                    1230

        TAT TCC AAC CAG CCC TTC ACA CCA CCG GAC TCC GTA GAT AAA GCG CTG 3744
        Tyr Ser Asn Gln Pro Phe Thr Pro Pro Asp Ser Val Asp Lys Ala Leu
                        1235              1240              1245

 15     GCA TTA ACC GGC GCA CTC CCT GTT GCC CAA TGT TTA GTC TAT GCC GTT 3792
        Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val
                    1250              1255              1260

        GAT AGC TGG ATG CCG TCG TTA TCT TTG TCT CAG CTT TCT CAG TCA CAA 3840
 20     Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln
        1265                1270              1275                    1280

        GAA GAG GCA GAA GCG CTA TGG GCG CAA CTG CGT GCC GCT CAT ATG ATT 3888
        Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile
 25                     1285              1290                    1295

        ACC GAA GAT GGG AAA GTG TGT GCG TTA AGC GGG AAA CGA GGA ACA AGC 3936
        Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser
                        1300              1305                    1310
 30
        CAT CAG AAC CTG ACG ATT CAA CTT ATT TCG CTA TTG GCA AGT ATT CCC 3984
        His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro
                        1315              1320              1325

 35     CGT TTA CCG CCA CAT GTA CTG GGG ATC ACC ACT GAT CGC TAT GAT AGC 4032
        Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser
                    1330              1335              1340

        GAT CCG CAA CAG CAG CAC CAA CAG ACG GTG AGC TTT AGT GAC GGT TTT 4080
 40     Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe
        1345                1350              1355                    1360

        GGC CGG TTA CTC CAG AGT TCA GCT CGT CAT GAG TCA GGT GAT GCC TGG 4128
        Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp
 45                     1365              1370              1375

        CAA CGT AAA GAG GAT GGC GGG CTG GTC GTG GAT GCA AAT GGC GTT CTG 4176
        Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu
                    1380              1385              1390
 50
        GTC AGT GCC CCT ACA GAC ACC CGA TGG GCC GTT TCC GGT CGC ACA GAA 4224
        Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu
                    1395              1400              1405

 55     TAT GAC GAC AAA GGC CAA CCT GTG CGT ACT TAT CAA CCC TAT TTT CTA 4272
        Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu
                1410              1415              1420

        AAT GAC TGG CGT TAC GTT AGT GAT GAC AGC GCA CGA GAT GAC CTG TTT 4320
 60     Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe
        1425              1430              1435                    1440

        GCC GAT ACC CAC CTT TAT GAT CCA TTG GGA CGG GAA TAC AAA GTC ATC 4368
        Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile
 65                     1445              1450              1455

        ACT GCT AAG AAA TAT TTG CGA GAA AAG CTG TAC ACC CCG TGG TTT ATT 4416
        Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile
                    1460              1465              1470
 70
        GTC AGT GAG GAT GAA AAC GAT ACA GCA TCA AGA ACC CCA TAG        4458
        Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro ***
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SUBSTITUTE SHEET (RULE 26)

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        Val Ser Glu Asp Glu Asn Asp Thr Ala Ser.Arg Thr Pro   *
            1475                1480                1485
```

5   (2) INFORMATION FOR SEQ ID NO:32:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 1485 amino acids
            (B) TYPE: amino acid
10   (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32 (TcaC protein):

```
15  Met Gln Asp Ser Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys
     1           5               10              15

    Gly Gly Gly Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly
                20              25              30
20
    Pro Asp Gly Met Ala Ser Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly
                35              40              45

    Arg Gly Thr Ala Pro Gly Leu Ser Leu Ile Tyr Ser Asn Ser Ala Gly
25      50              55              60

    Asn Gly Pro Phe Gly Ile Gly Trp Gln Cys Gly Val Met Ser Ile Ser
     65              70              75              80

30  Arg Arg Thr Gln His Gly Ile Pro Gln Tyr Gly Asn Asp Asp Thr Phe
                85              90              95

    Leu Ser Pro Gln Gly Glu Val Met Asn Ile Ala Leu Asn Asp Gln Gly
                100             105             110
35
    Gln Pro Asp Ile Arg Gln Asp Val Lys Thr Leu Gln Gly Val Thr Leu
                115             120             125

    Pro Ile Ser Tyr Thr Val Thr Arg Tyr Gln Ala Arg Gln Ile Leu Asp
40      130             135             140

    Phe Ser Lys Ile Glu Tyr Trp Gln Pro Ala Ser Gly Gln Glu Gly Arg
    145             150             155             160

45  Ala Phe Trp Leu Ile Ser Thr Pro Asp Gly His Leu His Ile Leu Gly
                165             170             175

    Lys Thr Ala Gln Ala Cys Leu Ala Asn Pro Gln Asn Asp Gln Gln Ile
                180             185             190
50
    Ala Gln Trp Leu Leu Glu Glu Thr Val Thr Pro Ala Gly Glu His Val
                195             200             205

    Ser Tyr Gln Tyr Arg Ala Glu Asp Glu Ala His Cys Asp Asp Asn Glu
55      210             215             220

    Lys Thr Ala His Pro Asn Val Thr Ala Gln Arg Tyr Leu Val Gln Val
    225             230             235             240

60  Asn Tyr Gly Asn Ile Lys Pro Gln Ala Ser Leu Phe Val Leu Asp Asn
                245             250             255

    Ala Pro Pro Ala Pro Glu Glu Trp Leu Phe His Leu Val Phe Asp His
                260             265             270
65
    Gly Glu Arg Asp Thr Ser Leu His Thr Val Pro Thr Trp Asp Ala Gly
                275             280             285

    Thr Ala Gln Trp Ser Val Arg Pro Asp Ile Phe Ser Arg Tyr Glu Tyr
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SUBSTITUTE SHEET (RULE 26)

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            290                   295                   300

      Gly Phe Glu Val Arg Thr Arg Arg Leu Cys Gln Gln Val Leu Met Phe
      305             310             315             320
  5
      His Arg Thr Ala Leu Met Ala Gly Glu Ala Ser Thr Asn Asp Ala Pro
                      325             330             335

      Glu Leu Val Gly Arg Leu Ile Leu Glu Tyr Asp Lys Asn Ala Ser Val
 10                   340             345             350

      Thr Thr Leu Ile Thr Ile Arg Gln Leu Ser His Glu Ser Asp Gly Arg
                      355             360             365

 15   Pro Val Thr Gln Pro Pro Leu Glu Leu Ala Trp Gln Arg Phe Asp Leu
                      370             375             380

      Glu Lys Ile Pro Thr Trp Gln Arg Phe Asp Ala Leu Asp Asn Phe Asn
      385             390             395             400
 20
      Ser Gln Gln Arg Tyr Gln Leu Val Asp Leu Arg Gly Glu Gly Leu Pro
                      405             410             415

      Gly Met Leu Tyr Gln Asp Arg Gly Ala Trp Trp Tyr Lys Ala Pro Gln
 25                   420             425             430

      Arg Gln Glu Asp Gly Asp Ser Asn Ala Val Thr Tyr Asp Lys Ile Ala
                      435             440             445

 30   Pro Leu Pro Thr Leu Pro Asn Leu Gln Asp Asn Ala Ser Leu Met Asp
                      450             455             460

      Ile Asn Gly Asp Gly Gln Leu Asp Trp Val Val Thr Ala Ser Gly Ile
      465             470             475             480
 35
      Arg Gly Tyr His Ser Gln Gln Pro Asp Gly Lys Trp Thr His Phe Thr
                      485             490             495

 40   Pro Ile Asn Ala Leu Pro Val Glu Tyr Phe His Pro Ser Ile Gln Phe
                      500             505             510

      Ala Asp Leu Thr Gly Ala Gly Leu Ser Asp Leu Val Leu Ile Gly Pro
                      515             520             525

 45   Lys Ser Val Arg Leu Tyr Ala Asn Gln Arg Asn Gly Trp Arg Lys Gly
                      530             535             540

      Glu Asp Val Pro Gln Ser Thr Gly Ile Thr Leu Pro Val Thr Gly Thr
      545             550             555             560
 50
      Asp Ala Arg Lys Leu Val Ala Phe Ser Asp Met Leu Gly Ser Gly Gln
                      565             570             575

 55   Gln His Leu Val Glu Ile Lys Gly Asn Arg Val Thr Cys Trp Pro Asn
                      580             585             590

      Leu Gly His Gly Arg Phe Gly Gln Pro Leu Thr Leu Ser Gly Phe Ser
                      595             600             605

 60   Gln Pro Glu Asn Ser Phe Asn Pro Glu Arg Leu Phe Leu Ala Asp Ile
                      610             615             620

      Asp Gly Ser Gly Thr Thr Asp Leu Ile Tyr Ala Gln Ser Gly Ser Leu
      625             630             635             640
 65
      Leu Ile Tyr Leu Asn Gln Ser Gly Asn Gln Phe Asp Ala Pro Leu Thr
                      645             650             655

 70   Leu Ala Leu Pro Glu Gly Val Gln Phe Asp Asn Thr Cys Gln Leu Gln
                      660             665             670
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SUBSTITUTE SHEET (RULE 26)

```
     Val Ala Asp Ile Gln Gly Leu Gly Ile Ala Ser Leu Ile Leu Thr Val
             675                 680             685

 5   Pro His Ile Ala Pro His His Trp Arg Cys Asp Leu Ser Leu Thr Lys
         690                 695             700

     Pro Trp Leu Leu Asn Val Met Asn Asn Asn Arg Gly Ala His His Thr
     705                 710             715                 720

10   Leu His Tyr Arg Ser Ser Ala Gln Phe Trp Leu Asp Glu Lys Leu Gln
                     725             730                 735

     Leu Thr Lys Ala Gly Lys Ser Pro Ala Cys Tyr Leu Pro Phe Pro Met
                 740             745             750

15   His Leu Leu Trp Tyr Thr Glu Ile Gln Asp Glu Ile Ser Gly Asn Arg
                 755             760             765

     Leu Thr Ser Glu Val Asn Tyr Ser His Gly Val Trp Asp Gly Lys Glu
20       770             775             780

     Arg Glu Phe Arg Gly Phe Gly Cys Ile Lys Gln Thr Asp Thr Thr Thr
     785             790             795             800

25   Phe Ser His Gly Thr Ala Pro Glu Gln Ala Ala Pro Ser Leu Ser Ile
                 805             810                 815

     Ser Trp Phe Ala Thr Gly Met Asp Glu Val Asp Ser Gln Leu Ala Thr
                 820             825             830

30   Glu Tyr Trp Gln Ala Asp Thr Gln Ala Tyr Ser Gly Phe Glu Thr Arg
                 835             840             845

     Tyr Thr Val Trp Asp His Thr Asn Gln Thr Asp Gln Ala Phe Thr Pro
35       850             855             860

     Asn Glu Thr Gln Arg Asn Trp Leu Thr Arg Ala Leu Lys Gly Gln Leu
     865             870             875             880

40   Leu Arg Thr Glu Leu Tyr Gly Leu Asp Gly Thr Asp Lys Gln Thr Val
                 885             890             895

     Pro Tyr Thr Val Ser Glu Ser Arg Tyr Gln Val Arg Ser Ile Pro Val
45               900             905             910

     Asn Lys Glu Thr Glu Leu Ser Ala Trp Val Thr Ala Ile Glu Asn Arg
                 915             920             925

50   Ser Tyr His Tyr Glu Arg Ile Ile Thr Asp Pro Gln Phe Ser Gln Ser
         930             935             940

     Ile Lys Leu Gln His Asp Ile Phe Gly Gln Ser Leu Gln Ser Val Asp
     945             950             955             960

55   Ile Ala Trp Pro Arg Arg Glu Lys Pro Ala Val Asn Pro Tyr Pro Pro
                 965             970             975

     Thr Leu Pro Glu Thr Leu Phe Asp Ser Ser Tyr Asp Asp Gln Gln Gln
60               980             985             990

     Leu Leu Arg Leu Val Arg Gln Lys Asn Ser Trp His His Leu Thr Asp
                 995             1000            1005

65   Gly Glu Asn Trp Arg Leu Gly Leu Pro Asn Ala Gln Arg Arg Asp Val
         1010            1015            1020

     Tyr Thr Tyr Asp Arg Ser Lys Ile Pro Thr Glu Gly Ile Ser Leu Glu
     1025            1030            1035            1040

70   Ile Leu Leu Lys Asp Asp Gly Leu Leu Ala Asp Glu Lys Ala Ala Val
                 1045            1050            1055
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SUBSTITUTE SHEET (RULE 26)

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        Tyr Leu Gly Gln Gln Gln Thr Phe Tyr Thr Ala Gly Gln Ala Glu Val
                    1060            1065            1070

 5      Thr Leu Glu Lys Pro Thr Leu Gln Ala Leu Val Ala Phe Gln Glu Thr
                    1075            1080                1085

        Ala Met Met Asp Asp Thr Ser Leu Gln Ala Tyr Glu Gly Val Ile Glu
                    1090            1095            1100
10
        Glu Gln Glu Leu Asn Thr Ala Leu Thr Gln Ala Gly Tyr Gln Gln Val
        1105            1110            1115                1120

15      Ala Arg Leu Phe Asn Thr Arg Ser Glu Ser Pro Val Trp Ala Ala Arg
                        1125            1130            1135

        Gln Gly Tyr Thr Asp Tyr Gly Asp Ala Ala Gln Phe Trp Arg Pro Gln
                    1140            1145            1150

20      Ala Gln Arg Asn Ser Leu Leu Thr Gly Lys Thr Thr Leu Thr Trp Asp
                    1155            1160            1165

        Thr His His Cys Val Ile Ile Gln Thr Gln Asp Ala Ala Gly Leu Thr
                    1170            1175            1180
25
        Thr Gln Ala His Tyr Asp Tyr Arg Phe Leu Thr Pro Val Gln Leu Thr
        1185            1190            1195                1200

        Asp Ile Asn Asp Asn Gln His Ile Val Thr Leu Asp Ala Leu Gly Arg
30                      1205            1210            1215

        Val Thr Thr Ser Arg Phe Trp Gly Thr Glu Ala Gly Gln Ala Ala Gly
                    1220            1225            1230

35      Tyr Ser Asn Gln Pro Phe Thr Pro Pro Asp Ser Val Asp Lys Ala Leu
                    1235            1240            1245

        Ala Leu Thr Gly Ala Leu Pro Val Ala Gln Cys Leu Val Tyr Ala Val
                    1250            1255            1260
40
        Asp Ser Trp Met Pro Ser Leu Ser Leu Ser Gln Leu Ser Gln Ser Gln
        1265            1270            1275                1280

        Glu Glu Ala Glu Ala Leu Trp Ala Gln Leu Arg Ala Ala His Met Ile
45                      1285            1290            1295

        Thr Glu Asp Gly Lys Val Cys Ala Leu Ser Gly Lys Arg Gly Thr Ser
                    1300            1305            1310

50      His Gln Asn Leu Thr Ile Gln Leu Ile Ser Leu Leu Ala Ser Ile Pro
                    1315            1320            1325

        Arg Leu Pro Pro His Val Leu Gly Ile Thr Thr Asp Arg Tyr Asp Ser
                    1330            1335            1340
55
        Asp Pro Gln Gln Gln His Gln Gln Thr Val Ser Phe Ser Asp Gly Phe
        1345            1350            1355                1360

        Gly Arg Leu Leu Gln Ser Ser Ala Arg His Glu Ser Gly Asp Ala Trp
60                      1365            1370            1375

        Gln Arg Lys Glu Asp Gly Gly Leu Val Val Asp Ala Asn Gly Val Leu
                    1380            1385            1390

65      Val Ser Ala Pro Thr Asp Thr Arg Trp Ala Val Ser Gly Arg Thr Glu
                    1395            1400            1405

        Tyr Asp Asp Lys Gly Gln Pro Val Arg Thr Tyr Gln Pro Tyr Phe Leu
                    1410            1415            1420
70
        Asn Asp Trp Arg Tyr Val Ser Asp Asp Ser Ala Arg Asp Asp Leu Phe
```

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      1425                1430              . 1435              1440
      Ala Asp Thr His Leu Tyr Asp Pro Leu Gly Arg Glu Tyr Lys Val Ile
                        1445              1450              1455
 5
      Thr Ala Lys Lys Tyr Leu Arg Glu Lys Leu Tyr Thr Pro Trp Phe Ile
                    1460              1465              1470

      Val Ser Glu Asp Glu Asn Asp Thr Ala Ser Arg Thr Pro  *
10             1475              1480              1485
```

(2) INFORMATION FOR SEQ ID NO:33:

15   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 3288 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
20   (ii) MOLECULE TYPE: DNA (genomic)

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33 (tcaA gene):

```
      ATG GTG ACT GTT ATG CAA AAT AAA ATA TCA TTT TTA TCA GGT ACA TCC  48
25    Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser
      1               5               10              15

      GAA CAG CCC CTG CTT GAC GCC GGT TAT CAA AAC GTA TTT GAT ATC GCA  96
      Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala
30                  20              25              30

      TCA ATC AGC CGG GCT ACT TTC GTT CAA TCC GTT CCC ACC CTG CCC GTT  144
      Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val
35                35              40              45

      AAA GAG GCT CAT ACC GTC TAT CGT CAG GCG CGG CAA CGT GCG GAA AAT  192
      Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn
                  50              55              60

40    CTG AAA TCC CTC TAC CGA GCC TGG CAA TTG CGT CAG GAG CCG GTT ATT  240
      Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile
      65              70              75              80

      AAA GGG CTG GCT AAA CTT AAC CTA CAA TCC AAC GTT TCT GTG CTT CAA  288
45    Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln
                      85              90              95

      GAT GCT TTG GTA GAG AAT ATT GGC GGT GAT GGG GAT TTC AGC GAT TTA  336
      Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu
50                  100             105             110

      ATG AAC CGT GCC AGT CAA TAT GCT GAC GCT GCC TCT ATT CAA TCC CTA  384
      Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu
55                  115             120             125

      TTT TCA CCG GGC CGT TAT GCT TCC GCA CTC TAC AGA GTT GCT AAA GAT  432
      Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp
                  130             135             140

60    CTG CAT AAA TCA GAT TCC AGT TTG CAT ATT GAT AAT CGC CGC GCT GAT  480
      Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp
      145             150             155             160

      CTG AAG GAT CTG ATA TTA AGC GAA ACG ACG ATG AAT AAA GAG GTC ACT  528
65    Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr
                  165             170             175

      TCC CTT GAT ATC TTG TTG GAT GTG CTA CAA AAA GGC GGT AAA GAT ATT  576
      Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile
```

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                    180                  185                    190
      ACT GAG CTG TCC GGC GCA TTC TTC CCA ATG ACG TTA CCT TAT GAC GAT  624
      Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp
 5            195                 200                  205

      CAT CTG TCG CAA ATC GAT TCC GCT TTA TCG GCA CAA GCC AGA ACG CTG  672
      His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu
              210                 215                  220
10
      AAC GGT GTG TGG AAT ACT TTG ACA GAT ACC ACG GCA CAA GCG GTT TCA  720
      Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser
      225                 230                  235                  240

15    GAA CAA ACC AGT AAT ACG AAT ACA CGC AAA CTG TTC GCT GCC CAA GAT  768
      Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp
                          245                 250                  255

      GGT AAT CAA GAT ACA TTT TTT TCC GGA AAC ACT TTT TAT TTC AAA GCG  816
20    Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala
                          260                 265                  270

      GTG GGA TTC AGC GGG CAA CCT ATG GTT TAC CTG TCA CAG TAC ACC AGC  864
      Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser
25            275                 280                  285

      GGG AAC GGC ATT GTC GGC GCA CAA TTG ATT GCA GGT AAT CCA GAC CAA  912
      Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln
              290                 295                  300
30
      GCC GCC GCC GCA ATA GTC GCA CCG TTG AAA CTC ACT TGG TCA ATG GCA  960
      Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala
      305                 310                  315                  320

35    AAA CAG TGT TAC TAC CTC GTC GCT CCC GAT GGT ACA ACG ATG GGA GAC  1008
      Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp
                          325                 330                  335

      GGT AAT GTT CTG ACC GGC TGT TTC TTA AGA GGC AAC AGC CCA ACT AAC  1056
40    Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn
                          340                 345                  350

      CCG GAT AAA GAC GGT ATT TTT GCT CAG GTA GCC AAC AAA TCA GGC AGT  1104
      Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser
45            355                 360                  365

      ACT CAG CCT TTG CCA AGC TTC CAT CTG CCG GTC ACA CTG GAA CAC AGC  1152
      Thr Gln Pro Leu Pro Ser Phe His Leu Pro Val Thr Leu Glu His Ser
              370                 375                  380
50
      GAG AAT AAA GAT CAG TAC TAT CTG AAA ACA GAG CAG GGT TAT ATC ACG  1200
      Glu Asn Lys Asp Gln Tyr Tyr Leu Lys Thr Glu Gln Gly Tyr Ile Thr
      385                 390                  395                  400

55    GTA GAT AGT TCC GGA CAG TCA AAT TGG AAA AAC GCG CTG GTT ATC AAT  1248
      Val Asp Ser Ser Gly Gln Ser Asn Trp Lys Asn Ala Leu Val Ile Asn
                          405                 410                  415

      GGG ACA AAA GAC AAG GGG CTG TTA TTA ACC TTT TGC AGC GAT AGC TCA  1296
60    Gly Thr Lys Asp Lys Gly Leu Leu Leu Thr Phe Cys Ser Asp Ser Ser
                          420                 425                  430

      GGC ACT CCG ACA AAC CCT GAT GAT GTG ATT CCT CCC GCT ATC AAT GAT  1344
      Gly Thr Pro Thr Asn Pro Asp Asp Val Ile Pro Pro Ala Ile Asn Asp
65            435                 440                  445

      ATT CCA TCG CCG CCA GCC CGC GAA ACA CTG TCA CTG ACG CCG GTC AGT  1392
      Ile Pro Ser Pro Pro Ala Arg Glu Thr Leu Ser Leu Thr Pro Val Ser
              450                 455                  460
70
      TAT CAA TTG ATG ACC AAT CCG GCA CCG ACA GAA GAT GAT ATT ACC AAC  1440
```

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```
        Tyr Gln Leu Met Thr Asn Pro Ala Pro Thr Glu Asp Asp Ile Thr Asn
        465             470             475             480

  5     CAT TAT GGT TTT AAC GGC GCT AGC TTA CGG GCT TCT CCA TTG TCA ACC   1488
        His Tyr Gly Phe Asn Gly Ala Ser Leu Arg Ala Ser Pro Leu Ser Thr
                        485             490             495

        AGC GAG TTG ACC AGC AAA CTG AAT TCT ATC GAT ACT TTC TGT GAG AAG   1536
 10     Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr Phe Cys Glu Lys
                    500             505             510

        ACC CGG TTA AGC TTC AAT CAG TTA ATG GAT TTG ACC GCT CAG CAA TCT   1584
        Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr Ala Gln Gln Ser
                    515             520             525
 15

        TAC AGT CAA AGC AGC ATT GAT GCG AAA GCA GCC AGC CGC TAT GTT CGT   1632
        Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser Arg Tyr Val Arg
            530             535             540

 20     TTT GGG GAA ACC ACC CCA ACC CGC GTC AAT GTC TAC GGT GCC GCT TAT   1680
        Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr Gly Ala Ala Tyr
        545             550             555             560

        CTG AAC AGC ACA CTG GCA GAC GCG GCT GAT GGT CAA TAT CTG TGG ATT   1728
 25     Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln Tyr Leu Trp Ile
                        565             570             575

        CAG ACT GAT GGC AAG AGC CTA AAT TTC ACT GAC GAT ACG GTA GTC GCC   1776
        Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp Thr Val Val Ala
 30                 580             585             590

        TTA GCC GGT CGC GCT GAA AAG CTG GTA CGT TTA TCA TCC CAG ACC GGG   1824
        Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser Ser Gln Thr Gly
                    595             600             605
 35

        CTA TCA TTT GAA GAA TTG GAC TGG CTG ATT GCC AAT GCC AGT CGT AGT   1872
        Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn Ala Ser Arg Ser
            610             615             620

 40     GTG CCG GAC CAC CAC GAC AAA ATT GTG CTG GAT AAG CCG GTC CTT GAA   1920
        Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys Pro Val Leu Glu
        625             630             635             640

        GCA CTG GCA GAG TAT GTC AGC CTA AAA CAG CGC TAT GGG CTT GAT GCC   1968
 45     Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala
                        645             650             655

        AAT ACC TTT GCG ACC TTC ATT AGT GCA GTA AAT CCT TAT ACG CCA GAT   2016
        Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp
 50                 660             665             670

        CAG ACA CCC AGT TTC TAT GAA ACC GCT TTC CGC TCT GCC GAC GGT AAT   2064
        Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn
                    675             680             685
 55

        CAT GTC ATT GCG CTA GGT ACA GAG GTG AAA TAT GCA GAA AAT GAG CAG   2112
        His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln
                    690             695             700

 60     GAT GAG TTA GCC GCC ATA TGC TGC AAA GCA TTG GGT GTC ACC AGT GAT   2160
        Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp
        705             710             715             720

        GAA CTG CTC CGT ATT GGT CGC TAT TGC TTC GGT AAT GCA GGC AGT TTT   2208
 65     Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe
                        725             730             735

        ACC TTG GAT GAA TAT ACC GCC AGT CAG TTG TAT CGC TTC GGC GCC ATT   2256
        Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile
 70                 740             745             750
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SUBSTITUTE SHEET (RULE 26)

```
     CCC CGT TTG TTT GGG CTG ACA TTT GCC CAA GCC GAA ATT TTA TGG CGT   2304
     Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg
             755                 760                 765

5    CTG ATG GAA GGC GGA AAA GAT ATC TTA TTG CAA CAG TTA GGT CAG GCA   2352
     Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala
             770                 775                 780

     AAA TCC CTG CAA CCA CTG GCT ATT TTA CGC CGT ACC GAG CAG GTG CTG   2400
10   Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu
     785                 790                 795                 800

     GAT TGG ATG TCG TCC GTA AAT CTA AGT CTG ACT TAT CTG CAA GGG ATG   2448
     Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met
15                       805                 810                 815

     GTA AGT ACG CAA TGG AGC GGT ACC GCC ACC GCT GAG ATG TTC AAT TTC   2496
     Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe
                         820                 825                 830
20
     TTG GAA AAC GTT TGT GAC AGC GTG AAT AGT CAA GCT GCC ACT AAA GAA   2544
     Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu
                 835                 840                 845

25   ACA ATG GAT TCG GCG TTA CAG CAG AAA GTG CTG CGG GCG CTA AGC GCC   2592
     Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala
             850                 855                 860

     GGT TTC GGC ATT AAG AGC AAT GTG ATG GGT ATC GTC ACC TTC TGG CTG   2640
30   Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu
     865                 870                 875                 880

     GAG AAA ATC ACA ATC GGT AGT GAT AAT CCT TTT ACA TTG GCA AAC TAC   2688
     Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr
35                       885                 890                 895

     TGG CAT GAT ATT CAA ACC CTG TTT AGC CAT GAC AAT GCC ACG TTA GAG   2736
     Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu
                         900                 905                 910
40
     TCC TTA CAA ACC GAC ACT TCT CTG GTA ATT GCT ACT CAG CAA CTT AGC   2784
     Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser
                 915                 920                 925

45   CAG CTA GTG TTA ATT GTG AAA TGG CTG AGC CTG ACC GAG CAG GAT CTG   2832
     Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu
             930                 935                 940

     CAA TTA CTG ACA ACC TAT CCC GAA CGT TTA ATC AAC GGC ATC ACG AAT   2880
50   Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn
     945                 950                 955                 960

     GTT CCT GTA CCC AAT CCG GAG CTA TTA CTC ACG CTA TCA CGT TTT AAG   2928
     Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu Ser Arg Phe Lys
55                       965                 970                 975

     CAG TGG GAA ACT CAA GTC ACC GTT TCC CGT GAT GAA GCG ATG CGC TGT   2976
     Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys
                         980                 985                 990
60
     TTC GAT CAA TTA AAT GCC AAT GAT ATG ACG ACT GAA AAT GCA GGT TCA   3024
     Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser
                 995                 1000                1005

65   CTG ATC GCC ACA TTG TAT GAG ATG GAT AAA GGT ACG GGA GCG CAA GTT   3072
     Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val
             1010                1015                1020

     AAT ACC TTG CTA TTA GGT GAA AAT AAC TGG CCG AAA AGT TTT ACC TCT   3120
70   Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser
     1025                1030                1035                1040
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```
CTC TGG CAA CTT CTG ACC TGG TTA CGC GTC GGG CAA AGA CTG AAT GTC   3168
Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val
              1045                    1050                1055

GGT AGT ACC ACT CTG GGC AAT CTG TTG TCC ATG ATG CAA GCA GAC CCT   3216
Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro
              1060                1065                1070

GCT GCC GAG AGT AGC GCT TTA TTG GCA TCA GTA GCC CAA AAC TTA AGT   3264
Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser
          1075                1080                1085

GCC GCA ATC AGC AAT CGT CAG TAA                                   3288
Ala Ala Ile Ser Asn Arg Gln •••
          1090                1095
```

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 1095 amino acids
       (B) TYPE: amino acids
       (C) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34 (TcaA protein):
    Features   From   To   Description
             254   267   SEQ ID NO:15
             254   492   TcaA$_{ii}$ peptide

```
Met Val Thr Val Met Gln Asn Lys Ile Ser Phe Leu Ser Gly Thr Ser
1               5               10              15

Glu Gln Pro Leu Leu Asp Ala Gly Tyr Gln Asn Val Phe Asp Ile Ala
          20              25              30

Ser Ile Ser Arg Ala Thr Phe Val Gln Ser Val Pro Thr Leu Pro Val
          35              40              45

Lys Glu Ala His Thr Val Tyr Arg Gln Ala Arg Gln Arg Ala Glu Asn
      50              55              60

Leu Lys Ser Leu Tyr Arg Ala Trp Gln Leu Arg Gln Glu Pro Val Ile
65              70              75              80

Lys Gly Leu Ala Lys Leu Asn Leu Gln Ser Asn Val Ser Val Leu Gln
              85              90              95

Asp Ala Leu Val Glu Asn Ile Gly Gly Asp Gly Asp Phe Ser Asp Leu
          100             105             110

Met Asn Arg Ala Ser Gln Tyr Ala Asp Ala Ala Ser Ile Gln Ser Leu
          115             120             125

Phe Ser Pro Gly Arg Tyr Ala Ser Ala Leu Tyr Arg Val Ala Lys Asp
      130             135             140

Leu His Lys Ser Asp Ser Ser Leu His Ile Asp Asn Arg Arg Ala Asp
145             150             155             160

Leu Lys Asp Leu Ile Leu Ser Glu Thr Thr Met Asn Lys Glu Val Thr
              165             170             175

Ser Leu Asp Ile Leu Leu Asp Val Leu Gln Lys Gly Gly Lys Asp Ile
              180             185             190

Thr Glu Leu Ser Gly Ala Phe Phe Pro Met Thr Leu Pro Tyr Asp Asp
```

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                    195                  200                    205

        His Leu Ser Gln Ile Asp Ser Ala Leu Ser Ala Gln Ala Arg Thr Leu
 5          210             215                 220

        Asn Gly Val Trp Asn Thr Leu Thr Asp Thr Thr Ala Gln Ala Val Ser
 10     225             230             235                     240

        Glu Gln Thr Ser Asn Thr Asn Thr Arg Lys Leu Phe Ala Ala Gln Asp
 10                     245             250                 255

        Gly Asn Gln Asp Thr Phe Phe Ser Gly Asn Thr Phe Tyr Phe Lys Ala
                        260             265                 270

 15     Val Gly Phe Ser Gly Gln Pro Met Val Tyr Leu Ser Gln Tyr Thr Ser
                275             280                 285

        Gly Asn Gly Ile Val Gly Ala Gln Leu Ile Ala Gly Asn Pro Asp Gln
                290             295                 300
 20
        Ala Ala Ala Ala Ile Val Ala Pro Leu Lys Leu Thr Trp Ser Met Ala
        305             310                 315                     320

        Lys Gln Cys Tyr Tyr Leu Val Ala Pro Asp Gly Thr Thr Met Gly Asp
 25                     325             330                 335

        Gly Asn Val Leu Thr Gly Cys Phe Leu Arg Gly Asn Ser Pro Thr Asn
                340             345                 350

 30     Pro Asp Lys Asp Gly Ile Phe Ala Gln Val Ala Asn Lys Ser Gly Ser
                355             360                 365

        Thr Gln Pro Leu Pro Ser Phe His Leu Pro Val Thr Leu Glu His Ser
                370             375                 380
 35
        Glu Asn Lys Asp Gln Tyr Tyr Leu Lys Thr Glu Gln Gly Tyr Ile Thr
        385                 390                 395                     400

 40     Val Asp Ser Ser Gly Gln Ser Asn Trp Lys Asn Ala Leu Val Ile Asn
                        405             410                     415

        Gly Thr Lys Asp Lys Gly Leu Leu Leu Thr Phe Cys Ser Asp Ser Ser
                        420             425                 430

 45     Gly Thr Pro Thr Asn Pro Asp Asp Val Ile Pro Pro Ala Ile Asn Asp
                435             440                 445

        Ile Pro Ser Pro Pro Ala Arg Glu Thr Leu Ser Leu Thr Pro Val Ser
                450             455                 460
 50
        Tyr Gln Leu Met Thr Asn Pro Ala Pro Thr Glu Asp Asp Ile Thr Asn
        465             470                 475                     480

        His Tyr Gly Phe Asn Gly Ala Ser Leu Arg Ala Ser Pro Leu Ser Thr
 55                     485                 490         W4 »     495

        Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr Phe Cys Glu Lys
                        500             505                 510

 60     Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr Ala Gln Gln Ser
                515                 520                 525

        Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser Arg Tyr Val Arg
                530             535                 540
 65
        Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr Gly Ala Ala Tyr
        545             550                 555                     560

        Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln Tyr Leu Trp Ile
 70                     565                 570                 575
```

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```
        Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp Thr Val Val Ala
                    580                   585               590

    5   Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser Ser Gln Thr Gly
                    595               600               605

        Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn Ala Ser Arg Ser
                610               615               620

   10   Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys Pro Val Leu Glu
        625               630               635               640

        Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr Gly Leu Asp Ala
                        645               650               655

   15   Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro Tyr Thr Pro Asp
                    660               665               670

        Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser Ala Asp Gly Asn
                675               680               685

   20   His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala Glu Asn Glu Gln
            690               695               700

        Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly Val Thr Ser Asp
   25   705               710               715               720

        Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn Ala Gly Ser Phe
                    725               730               735

   30   Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg Phe Gly Ala Ile
                    740               745               750

        Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu Ile Leu Trp Arg
   35               755               760               765

        Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln Leu Gly Gln Ala
                770               775               780

   40   Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr Glu Gln Val Leu
        785               790               795               800

        Asp Trp Met Ser Ser Val Asn Leu Ser Leu Thr Tyr Leu Gln Gly Met
                        805               810               815

   45   Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu Met Phe Asn Phe
                    820               825               830

        Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala Ala Thr Lys Glu
   50           835               840               845

        Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg Ala Leu Ser Ala
                850               855               860

   55   Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val Thr Phe Trp Leu
        865               870               875               880

        Glu Lys Ile Thr Ile Gly Ser Asp Asn Pro Phe Thr Leu Ala Asn Tyr
   60                   885               890               895

        Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn Ala Thr Leu Glu
                    900               905               910

   65   Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr Gln Gln Leu Ser
                915               920               925

        Gln Leu Val Leu Ile Val Lys Trp Leu Ser Leu Thr Glu Gln Asp Leu
   70           930               935               940

        Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn Gly Ile Thr Asn
        945               950               955               960
```

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```
        Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu Ser Arg Phe Lys
                    965             970                 975

  5     Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu Ala Met Arg Cys
                    980             985             990

        Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu Asn Ala Gly Ser
                    995            1000            1005
 10
        Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr Gly Ala Gln Val
                   1010            1015            1020

 15     Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys Ser Phe Thr Ser
        1025            1030            1035            1040

        Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln Arg Leu Asn Val
                   1045            1050            1055

 20     Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met Gln Ala Asp Pro
                   1060            1065            1070

        Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala Gln Asn Leu Ser
                   1075            1080            1085
 25
        Ala Ala Ile Ser Asn Arg Gln •••
        1090            1095
```

 30   (2) INFORMATION FOR SEQ ID NO:35

             (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 603 amino acids
                  (B) TYPE: amino acid
 35   (C) TOPOLOGY: linear
             (ii) MOLECULE TYPE: protein

             (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35 (TcaA$_{iii}$ protein):

```
 40     Pro Leu Ser Thr Ser Glu Leu Thr Ser Lys Leu Asn Ser Ile Asp Thr
        1               5               10                  15

        Phe Cys Glu Lys Thr Arg Leu Ser Phe Asn Gln Leu Met Asp Leu Thr
                    20              25              30
 45
        Ala Gln Gln Ser Tyr Ser Gln Ser Ser Ile Asp Ala Lys Ala Ala Ser
                    35              40              45

 50     Arg Tyr Val Arg Phe Gly Glu Thr Thr Pro Thr Arg Val Asn Val Tyr
            50              55              60

        Gly Ala Ala Tyr Leu Asn Ser Thr Leu Ala Asp Ala Ala Asp Gly Gln
        65              70              75              80

 55     Tyr Leu Trp Ile Gln Thr Asp Gly Lys Ser Leu Asn Phe Thr Asp Asp
                        85              90              95

        Thr Val Val Ala Leu Ala Gly Arg Ala Glu Lys Leu Val Arg Leu Ser
                   100             105             110
 60
        Ser Gln Thr Gly Leu Ser Phe Glu Glu Leu Asp Trp Leu Ile Ala Asn
                   115             120             125

        Ala Ser Arg Ser Val Pro Asp His His Asp Lys Ile Val Leu Asp Lys
 65                130             135             140

        Pro Val Leu Glu Ala Leu Ala Glu Tyr Val Ser Leu Lys Gln Arg Tyr
        145             150             155             160
```

SUBSTITUTE SHEET (RULE 26)

```
      Gly Leu Asp Ala Asn Thr Phe Ala Thr Phe Ile Ser Ala Val Asn Pro
                  165             170             175
   5  Tyr Thr Pro Asp Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe Arg Ser
                  180             185             190
      Ala Asp Gly Asn His Val Ile Ala Leu Gly Thr Glu Val Lys Tyr Ala
                  195             200             205
  10  Glu Asn Glu Gln Asp Glu Leu Ala Ala Ile Cys Cys Lys Ala Leu Gly
                  210             215             220
      Val Thr Ser Asp Glu Leu Leu Arg Ile Gly Arg Tyr Cys Phe Gly Asn
      225             230             235             240
  15  Ala Gly Arg Phe Thr Leu Asp Glu Tyr Thr Ala Ser Gln Leu Tyr Arg
                  245             250             255
  20  Phe Gly Ala Ile Pro Arg Leu Phe Gly Leu Thr Phe Ala Gln Ala Glu
                  260             265             270
      Ile Leu Trp Arg Leu Met Glu Gly Gly Lys Asp Ile Leu Leu Gln Gln
                  275             280             285
  25  Xxx Gly Gln Ala Lys Ser Leu Gln Pro Leu Ala Ile Leu Arg Arg Thr
                  290             295             300
      Glu Gln Val Leu Asp Trp Met Ser Pro Val Asn Leu Ser Leu Thr Tyr
      305             310             315             320
  30  Leu Gln Gly Met Val Ser Thr Gln Trp Ser Gly Thr Ala Thr Ala Glu
                  325             330             335
  35  Met Phe Asn Phe Leu Glu Asn Val Cys Asp Ser Val Asn Ser Gln Ala
                  340             345             350
      Xxx Thr Lys Glu Thr Met Asp Ser Ala Leu Gln Gln Lys Val Leu Arg
                  355             360             365
  40  Ala Leu Ser Ala Gly Phe Gly Ile Lys Ser Asn Val Met Gly Ile Val
                  370             375             380
      Thr Phe Trp Leu Glu Lys Ile Thr Ile Gly Arg Asp Asn Pro Phe Thr
      385             390             395             400
  45  Leu Ala Asn Tyr Trp His Asp Ile Gln Thr Leu Phe Ser His Asp Asn
                  405             410             415
  50  Ala Thr Leu Glu Ser Leu Gln Thr Asp Thr Ser Leu Val Ile Ala Thr
                  420             425             430
      Gln Gln Leu Ser Gln Leu Val Leu Ile Val Lys Trp Val Ser Leu Thr
                  435             440             445
  55  Glu Gln Asp Leu Gln Leu Leu Thr Thr Tyr Pro Glu Arg Leu Ile Asn
                  450             455             460
      Gly Ile Thr Asn Val Pro Val Pro Asn Pro Glu Leu Leu Leu Thr Leu
      465             470             475             480
  60  Ser Arg Phe Lys Gln Trp Glu Thr Gln Val Thr Val Ser Arg Asp Glu
                  485             490             495
  65  Ala Met Arg Cys Phe Asp Gln Leu Asn Ala Asn Asp Met Thr Thr Glu
                  500             505             510
      Asn Ala Gly Ser Leu Ile Ala Thr Leu Tyr Glu Met Asp Lys Gly Thr
                  515             520             525
  70  Gly Ala Gln Val Asn Thr Leu Leu Leu Gly Glu Asn Asn Trp Pro Lys
                  530             535             540
```

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```
        Ser Phe Thr Ser Leu Trp Gln Leu Leu Thr Trp Leu Arg Val Gly Gln
        545             550             555             560

  5     Arg Leu Asn Val Gly Ser Thr Thr Leu Gly Asn Leu Leu Ser Met Met
                    565             570             575

        Gln Ala Asp Pro Ala Ala Glu Ser Ser Ala Leu Leu Ala Ser Val Ala
                    580             585             590
 10

        Gln Asn Leu Ser Ala Ala Ile Ser Asn Arg Gln *
                    595             600
```

15   (2) INFORMATION FOR SEQ ID NO:36:

          (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 2557 base pairs
              (B) TYPE: nucleic acid
 20   (C) TOPOLOGY: linear
          (ii) MOLECULE TYPE: DNA (genomic)

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36 (*tcdA* internal
       fragment):
 25

```
       GAATTCGGCT TGCGTTTAAT ATTGATGATG TCTCGCTCTT CCGCCTGCTT AAAATTACCG   60
       ACCATGATAA TAAAGATGGA AAAATTAAAA ATAACCTAAA GAATCTTTCC AATTTATATA  120
       TTGGAAAATT ACTGGCAGAT ATTCATCAAT TAACCATTGA TGAACTGGAT TTATTACTGA  180
       TTGCCGTAGG TGAAGGAAAA ACTAATTTAT CCGCTATCAG TGATAAGCAA TTGGCTACCC  240
 30    TGATCAGAAA ACTCAATACT ATTACCAGCT GGCTACATAC ACAGAAGTGG AGTGTATTCC  300
       AGCTATTTAT CATGACCTCC ACCAGCTATA ACAAAACGCT AACGCCTGAA ATTAAGAATT  360
       TGCTGGATAC CGTCTACCAC GGTTTACAAG GTTTTGATAA AGACAAAGCA GATTTGCTAC  420
       ATGTCATGGC GCCCTATATT GCGGCCACCT TGCAATTATC ATCGGAAAAT GTCGCCCACT  480
       CGGTACTCCT TTGGGCAGAT AAGTTACAGC CCGGCGACGG CGCAATGACA GCAGAGGGAN  540
 35    TCTGGGACTG GTTGAATACT AAGTATACGC CGGGTTCATC GGAAGCCGTA GAAACGCAGG  600
       AACATATCGT TCAGTATTGT CAGGCTCTGG CACAATTGGA AATGGTTTAC CATTCCACCG  660
       GCATCAACGA AAACGCCTTC CGTCTATTTG TGACAAAACC AGAGATGTTT GGCGCTGCAA  720
       CTGGAGCAGC GCCCGCGCAT GATGCCCTTT CACTGATTAT GCTGACACGT TTTGCGGATT  780
       GGGTGAACGC ACTAGGCGAA AAAGCGTCCT CGGTGCTAGC GGCATTTGAA GCTAACTCGT  840
 40    TAACGGCAGA ACAACTGGCT GATGCCATGA ATCTTGATGC TAATTTGCTG TTGCAAGCCA  900
       GTATTCAAGC ACAAAATCAT CAACATCTTC CCCCAGTAAC TCCAGAAAAT GCGTTCTCCT  960
       GTTGGACATC TATCAATACT ATCCTGCAAT GGGTTAATGT CGCACAACAA TTGAAATGTC 1020
       GCCCCACAGG GCGTTTCCGC TTTGGTCGGG CTGGATTATA TTCAATCAAT GAAAGAGACA 1080
       CCGACCTATG CCCAGTGGGA AAACGCGGCA GGCGTATTAA CCGCCGGGTT GAATTCAACA 1140
 45    ACAGGCTAAT ACATTACAAC GCTTTTCTGG ATGAATCTCG CAGTGCCGCA TTAAGCACCT 1200
       ACTATATCCG TCAAGTCGCC AAGGCAGCGG CGGCTATTAA AGCCGTGAT GACTTGTATC 1260
       AATACTTACT GATTGATAAT CAGGTTTCTG CGGCAATAAA AACCACCCGG ATCGCCGAAG 1320
       CCATTGCCAG TATTCAACTG TACGTCAACC GGGCATTGGA AAATGTGGAA GAAAATGCCA 1380
       ATTCGGGGGT TATCAGCCGC CAATTCTTTA TCGACTGGGA CAAATACAAT AAACGCTACA 1440
 50    GCACTTGGGC GGGTGTTTCT CAATTAGTTT ACTACCCGGA AAACTATATT GATCCGACCA 1500
       TGCGTATCGG ACAAACCAAA ATGATGGACG CATTACTGCA ATCCGTCAGC CAAAGCCAAT 1560
       TAAACGCCGA TACCGTCGAA GATGCCTTTA TGTCTTATCT GACATCGTTT GAACAAGTGG 1620
       CTAATCTTAA AGTTATTAGC GCATATCACG ATAATATTAA TAACGATCAA GGGCTGACCT 1680
       ATTTTATCGG ACTCAGTGAA ACTGATGCCG GTGAATATTA TTGGCGCAGT GTCGATCACA 1740
 55    GTAAATTCAA CGACGGTAAA TTCGCGGCTA ATGCCTGGAG TGAATGGCAT AAAATTGATT 1800
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SUBSTITUTE SHEET (RULE 26)

```
GTCCAATTAA CCCTTATAAA AGCACTATCC GTCCAGTGAT ATATAAATCC CGCCTGTATC   1860
TGCTCTGGTT GGAACAAAAG GAGATCACCA AACAGACAGG AAATAGTAAA GATGGCTATC   1920
AAACTGAAAC GGATTATCGT TATGAACTAA AATTGGCGCA TATCCGCTAT GATGGCACTT   1980
GGAATACGCC AATCACCTTT GATGTCAATA AAAAAATATC CGAGCTAAAA CTGGAAAAAA   2040
```
ATAGAGCGCC CGGACTCTAT TGTGCCGGTT ATCAAGGTGA AGATACGTTG CTGGTGATGT   2100
```
TTTATAACCA ACAAGACACA CTAGATAGTT ATAAAAACGC TTCAATGCAA GGACTATATA   2160
TCTTTGCTGA TATGGCATCC AAAGATATGA CCCCAGAACA GAGCAATGTT TATCGGGATA   2220
ATAGCTATCA ACAATTTGAT ACCAATAATG TCAGAAGAGT GAATAACCGC TATGCAGAGG   2280
ATTATGAGAT TCCTTCTTCG GTAAGTAGCC GTAAAGACTA TGGTTGGGGA GATTATTACC   2340
```
TCAGCATGGT ATATAACGGA GATATTCCAA CTATCAATTA CAAAGCCGCA TCAAGTGATT   2400
```
TAAAAATTTA TATTTCACCA AAATTAAGAA TTATTCATAA TGGATATGAA GGACAGAAGC   2460
GCAATCAATG CAATTTGATG AATAAATATG GCAAACTAGG TGATAAATTT ATTGTGTATA   2520
CCAGCCTGGG CGTTAATCCG AATAATAAGC CGAATTC                           2557
```

(2) INFORMATION FOR SEQ ID NO:37:

    (i)   SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 845 amino acids
          (B) TYPE: amino acids
          (C) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein (partial)

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37 (TcdA internal
peptide):

```
Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile Thr
1               5                   10                  15

Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn Leu
                20                  25                  30

Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu Thr
        35                  40                  45

Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys Thr
        50                  55                  60

Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg Lys
65                  70                  75                  80

Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val Phe
                85                  90                  95

Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr Pro
            100                 105                 110

Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly Phe
            115                 120                 125

Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile Ala
        130                 135                 140

Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu Leu
145                 150                 155                 160

Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu Gly
                165                 170                 175

Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu Ala
                180                 185                 190

Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala Gln
```

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```
                  195                    200                    205

      Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe Arg
          210             215             220
  5
      Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala Ala
          225             230             235             240

      Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala Asp
 10                   245             250             255

      Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala Phe
                      260             265             270

 15   Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn Leu
              275             280             285

      Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His Gln
          290             295             300
 20
      His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr Ser
      305             310             315             320

      Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Lys Cys
 25                   325             330             335

      Arg Pro Thr Gly Arg Phe Arg Phe Gly Arg Ala Gly Leu Tyr Ser Ile
                  340             345             350

 30   Asn Glu Arg Asp Thr Asp Leu Cys Pro Val Gly Lys Arg Gly Arg Arg
              355             360             365

      Ile Asn Arg Arg Val Glu Phe Asn Asn Arg Leu Ile His Tyr Asn Ala
          370             375             380
 35
      Phe Leu Asp Glu Ser Arg Ser Ala Ala Leu Ser Thr Tyr Tyr Ile Arg
      385             390             395             400

      Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
 40               405             410             415

      Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
              420             425             430

 45   Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
              435             440             445

      Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
          450             455             460
 50
      Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
      465             470             475             480

      Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
 55               485             490             495

      Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
                  500             505             510

 60   Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
              515             520             525

      Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala
          530             535             540
 65
      Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
      545             550             555             560

      Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
 70               565             570             575
```

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SUBSTITUTE SHEET (RULE 26)

```
        Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
                580                     585                 590

   5    His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
                595                     600                 605

        Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
                610             615                 620

  10    Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
        625                 630                 635                 640

        Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
                        645                 650                 655

  15    Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
                    660                 665                 670

        Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
                675                 680                 685

  20    Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
                690             695                 700

        Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
  25    705                 710                 715                 720

        Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
                        725                 730                 735

  30    Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
                    740                 745                 750

        Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
  35            755                 760                 765

        Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp
                770             775                 780

  40    Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
        785                 790                 795                 800

        Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
                        805                 810                 815
  45
        Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
                    820                 825                 830

        Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn
  50            835                 840                 845
```

(2) INFORMATION FOR SEQ ID NO:38:

  55   (i) SEQUENCE CHARACTERISTICS:
                 (A)   LENGTH: 16 amino acids
                 (B)   TYPE: amino acid
                 (C)   STRANDEDNESS: single
                 (D)   TOPOLOGY: linear
  60   (ii) MOLECULAR TYPE: protein
          (v) FRAGMENT TYPE: N-terminal

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38 (TcdA$_{ii}$- pk71 internal
      peptide):
  65
      Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly
      1   5   10   15

SUBSTITUTE SHEET (RULE 26)

Lys

(2) INFORMATION FOR SEQ ID NO:39:

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 20 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
         (v) FRAGMENT TYPE: N-terminal

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39 (TcdA$_{ii}$- pK44 internal
peptide):

Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala
1   5   10   15

Ile Ser Pro Ala Lys
               20

(2) INFORMATION FOR SEQ ID NO:40:

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 11 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
         (v) FRAGMENT TYPE: N-terminal

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40 (TcbA$_{iii}$ N-terminus):

Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln
1   5   10

(2) INFORMATION FOR SEQ ID NO:41:

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 14 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
         (v) FRAGMENT TYPE: N-terminal

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41 (TcdA$_{iii}$ N-terminus):

Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln
1   5   10

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        (2) INFORMATION FOR SEQ ID NO:42:

              (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 19 amino acids
  5   (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear
              (ii) MOLECULAR TYPE: protein
              (v) FRAGMENT TYPE: N-terminal
 10
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42 (TcdA-pk57 internal
peptide):

Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu Ala Glu Val Tyr
 15 1   5   10   15

Ala Gly Leu Glu

 20   (2) INFORMATION FOR SEQ ID NO:43:

              (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 11 amino acids
                    (B) TYPE: amino acid
 25   (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear
              (ii) MOLECULAR TYPE: protein
              (v) FRAGMENT TYPE: N-terminal

 30   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43 (TcdA$_{iii}$-pK20 internal
peptide):

Ile Arg Glu Asp Tyr Pro Ala Ser Leu Gly Lys
 35 1   5   10

        (2) INFORMATION FOR SEQ ID NO:44:

              (i) SEQUENCE CHARACTERISTICS:
 40   (A) LENGTH: 16 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear
              (ii) MOLECULAR TYPE: protein
 45   (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Asp Ser Gly Asp Asp Asp Lys Val Thr Asn Thr Asp Ile His Arg
 50 1   5   10   15

        (2) INFORMATION FOR SEQ ID NO:45:

 55   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 13 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear
 60   (ii) MOLECULAR TYPE: protein
              (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

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```
        Asp Val Xaa Gly Ser Glu Lys Ala Asn Glu Lys Leu Lys
        1           5                   10
```

5   (2) INFORMATION FOR SEQ ID NO:46:

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 7551 base pairs
              (B) TYPE: nucleic acid
10   (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear
         (ii) MOLECULE TYPE: DNA (genomic)

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46 (tcdA):
15
```
        ATG AAC GAG TCT GTA AAA GAG ATA CCT GAT GTA TTA AAA AGC CAG TGT   48
        Met Asn Glu Ser Val Lys Glu Ile Pro Asp Val Leu Lys Ser Gln Cys
        1           5                   10                  15

20      GGT TTT AAT TGT CTG ACA GAT ATT AGC CAC AGC TCT TTT AAT GAA TTT   96
        Gly Phe Asn Cys Leu Thr Asp Ile Ser His Ser Ser Phe Asn Glu Phe
                    20                  25                  30

        CGC CAG CAA GTA TCT GAG CAC CTC TCC TGG TCC GAA ACA CAC GAC TTA   144
25      Arg Gln Gln Val Ser Glu His Leu Ser Trp Ser Glu Thr His Asp Leu
                    35                  40                  45

        TAT CAT GAT GCA CAA CAG GCA CAA AAG GAT AAT CGC CTG TAT GAA GCG   192
        Tyr His Asp Ala Gln Gln Ala Gln Lys Asp Asn Arg Leu Tyr Glu Ala
30          50                  55                  60

        CGT ATT CTC AAA CGC GCC AAT CCC CAA TTA CAA AAT GCG GTG CAT CTT   240
        Arg Ile Leu Lys Arg Ala Asn Pro Gln Leu Gln Asn Ala Val His Leu
        65                  70                  75                  80
35
        GCC ATT CTC GCT CCC AAT GCT GAA CTG ATA GGC TAT AAC AAT CAA TTT   288
        Ala Ile Leu Ala Pro Asn Ala Glu Leu Ile Gly Tyr Asn Asn Gln Phe
                            85                  90                  95

40      AGC GGT AGA GCC AGT CAA TAT GTT GCG CCG GGT ACC GTT TCT TCC ATG   336
        Ser Gly Arg Ala Ser Gln Tyr Val Ala Pro Gly Thr Val Ser Ser Met
                        100                 105                 110

        TTC TCC CCC GCC GCT TAT TTG ACT GAA CTT TAT CGT GAA GCA CGC AAT   384
45      Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Arg Asn
                    115                 120                 125

        TTA CAC GCA AGT GAC TCC GTT TAT TAT CTG GAT ACC CGC CGC CCA GAT   432
        Leu His Ala Ser Asp Ser Val Tyr Tyr Leu Asp Thr Arg Arg Pro Asp
50          130                 135                 140

        CTC AAA TCA ATG GCG CTC AGT CAG CAA AAT ATG GAT ATA GAA TTA TCC   480
        Leu Lys Ser Met Ala Leu Ser Gln Gln Asn Met Asp Ile Glu Leu Ser
        145                 150                 155                 160
55
        ACA CTC TCT TTG TCC AAT GAG CTG TTA TTG GAA AGC ATT AAA ACT GAA   528
        Thr Leu Ser Leu Ser Asn Glu Leu Leu Leu Glu Ser Ile Lys Thr Glu
                        165                 170                 175

60      TCT AAA CTG GAA AAC TAT ACT AAA GTG ATG GAA ATG CTC TCC ACT TTC   576
        Ser Lys Leu Glu Asn Tyr Thr Lys Val Met Glu Met Leu Ser Thr Phe
                    180                 185                 190

        CGT CCT TCC GGC GCA ACG CCT TAT CAT GAT GCT TAT GAA AAT GTG CGT   624
65      Arg Pro Ser Gly Ala Thr Pro Tyr His Asp Ala Tyr Glu Asn Val Arg
                    195                 200                 205

        GAA GTT ATC CAG CTA CAA GAT CCT GGA CTT GAG CAA CTC AAT GCA TCA   672
        Glu Val Ile Gln Leu Gln Asp Pro Gly Leu Glu Gln Leu Asn Ala Ser
```

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              210                    215                    220

    CCG GCA ATT GCC GGG TTG ATG CAT CAA GCC TCC CTA TTG GGT ATT AAC   720
    Pro Ala Ile Ala Gly Leu Met His Gln Ala Ser Leu Leu Gly Ile Asn
5   225                 230                 235                 240

    GCT TCA ATC TCG CCT GAG CTA TTT AAT ATT CTG ACG GAG GAG ATT ACC   768
    Ala Ser Ile Ser Pro Glu Leu Phe Asn Ile Leu Thr Glu Glu Ile Thr
10                      245                 250                 255

    GAA GGT AAT GCT GAG GAA CTT TAT AAG AAA AAT TTT GGT AAT ATC GAA   816
    Glu Gly Asn Ala Glu Glu Leu Tyr Lys Lys Asn Phe Gly Asn Ile Glu
                        260                 265                 270

15  CCG GCC TCA TTG GCT ATG CCG GAA TAC CTT AAA CGT TAT TAT AAT TTA   864
    Pro Ala Ser Leu Ala Met Pro Glu Tyr Leu Lys Arg Tyr Tyr Asn Leu
                        275                 280                 285

    AGC GAT GAA GAA CTT AGT CAG TTT ATT GGT AAA GCC AGC AAT TTT GGT   912
20  Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly Lys Ala Ser Asn Phe Gly
        290                 295                 300

    CAA CAG GAA TAT AGT AAT AAC CAA CTT ATT ACT CCG GTA GTC AAC AGC   960
    Gln Gln Glu Tyr Ser Asn Asn Gln Leu Ile Thr Pro Val Val Asn Ser
25  305                 310                 315                 320

    AGT GAT GGC ACG GTT AAG GTA TAT CGG ATC ACC CGC GAA TAT ACA ACC   1008
    Ser Asp Gly Thr Val Lys Val Tyr Arg Ile Thr Arg Glu Tyr Thr Thr
                        325                 330                 335
30

    AAT GCT TAT CAA ATG GAT GTG GAG CTA TTT CCC TTC GGT GGT GAG AAT   1056
    Asn Ala Tyr Gln Met Asp Val Glu Leu Phe Pro Phe Gly Gly Glu Asn
                        340                 345                 350

35  TAT CGG TTA GAT TAT AAA TTC AAA AAT TTT TAT AAT GCC TCT TAT TTA   1104
    Tyr Arg Leu Asp Tyr Lys Phe Lys Asn Phe Tyr Asn Ala Ser Tyr Leu
                        355                 360                 365

    TCC ATC AAG TTA AAT GAT AAA AGA GAA CTT GTT CGA ACT GAA GGC GCT   1152
40  Ser Ile Lys Leu Asn Asp Lys Arg Glu Leu Val Arg Thr Glu Gly Ala
        370                 375                 380

    CCT CAA GTC AAT ATA GAA TAC TCC GCA AAT ATC ACA TTA AAT ACC GCT   1200
    Pro Gln Val Asn Ile Glu Tyr Ser Ala Asn Ile Thr Leu Asn Thr Ala
45  385                 390                 395                 400

    GAT ATC AGT CAA CCT TTT GAA ATT GGC CTG ACA CGA GTA CTT CCT TCC   1248
    Asp Ile Ser Gln Pro Phe Glu Ile Gly Leu Thr Arg Val Leu Pro Ser
                        405                 410                 415
50

    GGT TCT TGG GCA TAT GCC GCC GCA AAA TTT ACC GTT GAA GAG TAT AAC   1296
    Gly Ser Trp Ala Tyr Ala Ala Ala Lys Phe Thr Val Glu Glu Tyr Asn
                        420                 425                 430

55  CAA TAC TCT TTT CTG CTA AAA CTT AAC AAG GCT ATT CGT CTA TCA CGT   1344
    Gln Tyr Ser Phe Leu Leu Lys Leu Asn Lys Ala Ile Arg Leu Ser Arg
                        435                 440                 445

    GCG ACA GAA TTG TCA CCC ACG ATT CTG GAA GGC ATT GTG CGC AGT GTT   1392
60  Ala Thr Glu Leu Ser Pro Thr Ile Leu Glu Gly Ile Val Arg Ser Val
        450                 455                 460

    AAT CTA CAA CTG GAT ATC AAC ACA GAC GTA TTA GGT AAA GTT TTT CTG   1440
    Asn Leu Gln Leu Asp Ile Asn Thr Asp Val Leu Gly Lys Val Phe Leu
65  465                 470                 475                 480

    ACT AAA TAT TAT ATG CAG CGT TAT GCT ATT CAT GCT GAA ACT GCC CTG   1488
    Thr Lys Tyr Tyr Met Gln Arg Tyr Ala Ile His Ala Glu Thr Ala Leu
                        485                 490                 495
70

    ATA CTA TGC AAC GCG CCT ATT TCA CAA CGT TCA TAT GAT AAT CAA CCT   1536
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SUBSTITUTE SHEET (RULE 26)

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        Ile Leu Cys Asn Ala Pro Ile Ser Gln Arg Ser Tyr Asp Asn Gln Pro
                500                 505                 510

  5     AGC CAA TTT GAT CGC CTG TTT AAT ACG CCA TTA CTG AAC GGA CAA TAT   1584
        Ser Gln Phe Asp Arg Leu Phe Asn Thr Pro Leu Leu Asn Gly Gln Tyr
                515                 520                 525

        TTT TCT ACC GGC GAT GAG GAG ATT GAT TTA AAT TCA GGT AGC ACC GGC   1632
 10     Phe Ser Thr Gly Asp Glu Glu Ile Asp Leu Asn Ser Gly Ser Thr Gly
                530                 535                 540

        GAT TGG CGA AAA ACC ATA CTT AAG CGT GCA TTT AAT ATT GAT GAT GTC   1680
        Asp Trp Arg Lys Thr Ile Leu Lys Arg Ala Phe Asn Ile Asp Asp Val
        545                 550                 555                 560
 15
        TCG CTC TTC CGC CTG CTT AAA ATT ACC GAC CAT GAT AAT AAA GAT GGA   1728
        Ser Leu Phe Arg Leu Leu Lys Ile Thr Asp His Asp Asn Lys Asp Gly
                        565                 570                 575

 20     AAA ATT AAA AAT AAC CTA AAG AAT CTT TCC AAT TTA TAT ATT GGA AAA   1776
        Lys Ile Lys Asn Asn Leu Lys Asn Leu Ser Asn Leu Tyr Ile Gly Lys
                        580                 585                 590

        TTA CTG GCA GAT ATT CAT CAA TTA ACC ATT GAT GAA CTG GAT TTA TTA   1824
 25     Leu Leu Ala Asp Ile His Gln Leu Thr Ile Asp Glu Leu Asp Leu Leu
                595                 600                 605

        CTG ATT GCC GTA GGT GAA GGA AAA ACT AAT TTA TCC GCT ATC AGT GAT   1872
        Leu Ile Ala Val Gly Glu Gly Lys Thr Asn Leu Ser Ala Ile Ser Asp
 30             610                 615                 620

        AAG CAA TTG GCT ACC CTG ATC AGA AAA CTC AAT ACT ATT ACC AGC TGG   1920
        Lys Gln Leu Ala Thr Leu Ile Arg Lys Leu Asn Thr Ile Thr Ser Trp
        625                 630                 635                 640
 35
        CTA CAT ACA CAG AAG TGG AGT GTA TTC CAG CTA TTT ATC ATG ACC TCC   1968
        Leu His Thr Gln Lys Trp Ser Val Phe Gln Leu Phe Ile Met Thr Ser
                        645                 650                 655

 40     ACC AGC TAT AAC AAA ACG CTA ACG CCT GAA ATT AAG AAT TTG CTG GAT   2016
        Thr Ser Tyr Asn Lys Thr Leu Thr Pro Glu Ile Lys Asn Leu Leu Asp
                        660                 665                 670

        ACC GTC TAC CAC GGT TTA CAA GGT TTT GAT AAA GAC AAA GCA GAT TTG   2064
 45     Thr Val Tyr His Gly Leu Gln Gly Phe Asp Lys Asp Lys Ala Asp Leu
                        675                 680                 685

        CTA CAT GTC ATG GCG CCC TAT ATT GCG GCC ACC TTG CAA TTA TCA TCG   2112
        Leu His Val Met Ala Pro Tyr Ile Ala Ala Thr Leu Gln Leu Ser Ser
 50             690                 695                 700

        GAA AAT GTC GCC CAC TCG GTA CTC CTT TGG GCA GAT AAG TTA CAG CCC   2160
        Glu Asn Val Ala His Ser Val Leu Leu Trp Ala Asp Lys Leu Gln Pro
        705                 710                 715                 720
 55
        GGC GAC GGC GCA ATG ACA GCA GAA AAA TTC TGG GAC TGG TTG AAT ACT   2208
        Gly Asp Gly Ala Met Thr Ala Glu Lys Phe Trp Asp Trp Leu Asn Thr
                        725                 730                 735

 60     AAG TAT ACG CCG GGT TCA TCG GAA GCC GTA GAA ACG CAG GAA CAT ATC   2256
        Lys Tyr Thr Pro Gly Ser Ser Glu Ala Val Glu Thr Gln Glu His Ile
                        740                 745                 750

        GTT CAG TAT TGT CAG GCT CTG GCA CAA TTG GAA ATG GTT TAC CAT TCC   2304
 65     Val Gln Tyr Cys Gln Ala Leu Ala Gln Leu Glu Met Val Tyr His Ser
                755                 760                 765

        ACC GGC ATC AAC GAA AAC GCC TTC CGT CTA TTT GTG ACA AAA CCA GAG   2352
 70     Thr Gly Ile Asn Glu Asn Ala Phe Arg Leu Phe Val Thr Lys Pro Glu
                770                 775                 780
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       ATG TTT GGC GCT GCA ACT GGA GCA GCG CCC GCG CAT GAT GCC CTT TCA   2400
       Met Phe Gly Ala Ala Thr Gly Ala Ala Pro Ala His Asp Ala Leu Ser
       785             790             795             800

 5     CTG ATT ATG CTG ACA CGT TTT GCG GAT TGG GTG AAC GCA CTA GGC GAA   2448
       Leu Ile Met Leu Thr Arg Phe Ala Asp Trp Val Asn Ala Leu Gly Glu
                   805             810             815

       AAA GCG TCC TCG GTG CTA GCG GCA TTT GAA GCT AAC TCG TTA ACG GCA   2496
10     Lys Ala Ser Ser Val Leu Ala Ala Phe Glu Ala Asn Ser Leu Thr Ala
                   820             825             830

       GAA CAA CTG GCT GAT GCC ATG AAT CTT GAT GCT AAT TTG CTG TTG CAA   2544
       Glu Gln Leu Ala Asp Ala Met Asn Leu Asp Ala Asn Leu Leu Leu Gln
15                 835             840             845

       GCC AGT ATT CAA GCA CAA AAT CAT CAA CAT CTT CCC CCA GTA ACT CCA   2592
       Ala Ser Ile Gln Ala Gln Asn His Gln His Leu Pro Pro Val Thr Pro
                   850             855             860
20
       GAA AAT GCG TTC TCC TGT TGG ACA TCT ATC AAT ACT ATC CTG CAA TGG   2640
       Glu Asn Ala Phe Ser Cys Trp Thr Ser Ile Asn Thr Ile Leu Gln Trp
       865             870             875             880

25     GTT AAT GTC GCA CAA CAA TTG AAT GTC GCC CCA CAG GGC GTT TCC GCT   2688
       Val Asn Val Ala Gln Gln Leu Asn Val Ala Pro Gln Gly Val Ser Ala
                       885             890             895

       TTG GTC GGG CTG GAT TAT ATT CAA TCA ATG AAA GAG ACA CCG ACC TAT   2736
30     Leu Val Gly Leu Asp Tyr Ile Gln Ser Met Lys Glu Thr Pro Thr Tyr
                   900             905             910

       GCC CAG TGG GAA AAC GCG GCA GGC GTA TTA ACC GCC GGG TTG AAT TCA   2784
       Ala Gln Trp Glu Asn Ala Ala Gly Val Leu Thr Ala Gly Leu Asn Ser
35                 915             920             925

       CAA CAG GCT AAT ACA TTA CAC GCT TTT CTG GAT GAA TCT CGC AGT GCC   2832
       Gln Gln Ala Asn Thr Leu His Ala Phe Leu Asp Glu Ser Arg Ser Ala
                   930             935             940
40
       GCA TTA AGC ACC TAC TAT ATC CGT CAA GTC GCC AAG GCA GCG GCG GCT   2880
       Ala Leu Ser Thr Tyr Tyr Ile Arg Gln Val Ala Lys Ala Ala Ala Ala
       945             950             955             960

45     ATT AAA AGC CGT GAT GAC TTG TAT CAA TAC TTA CTG ATT GAT AAT CAG   2928
       Ile Lys Ser Arg Asp Asp Leu Tyr Gln Tyr Leu Leu Ile Asp Asn Gln
                   965             970             975

       GTT TCT GCG GCA ATA AAA ACC ACC CGG ATC GCC GAA GCC ATT GCC AGT   2976
50     Val Ser Ala Ala Ile Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Ser
                   980             985             990

       ATT CAA CTG TAC GTC AAC CGG GCA TTG GAA AAT GTG GAA GAA AAT GCC   3024
       Ile Gln Leu Tyr Val Asn Arg Ala Leu Glu Asn Val Glu Glu Asn Ala
55                 995             1000            1005

       AAT TCG GGG GTT ATC AGC CGC CAA TTC TTT ATC GAC TGG GAC AAA TAC   3072
       Asn Ser Gly Val Ile Ser Arg Gln Phe Phe Ile Asp Trp Asp Lys Tyr
       1010            1015            1020
60
       AAT AAA CGC TAC AGC ACT TGG GCG GGT GTT TCT CAA TTA GTT TAC TAC   3120
       Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Gln Leu Val Tyr Tyr
       1025            1030            1035            1040

65     CCG GAA AAC TAT ATT GAT CCG ACC ATG CGT ATC GGA CAA ACC AAA ATG   3168
       Pro Glu Asn Tyr Ile Asp Pro Thr Met Arg Ile Gly Gln Thr Lys Met
                   1045            1050            1055

       ATG GAC GCA TTA CTG CAA TCC GTC AGC CAA AGC CAA TTA AAC GCC GAT   3216
70     Met Asp Ala Leu Leu Gln Ser Val Ser Gln Ser Gln Leu Asn Ala Asp
                   1060            1065            1070
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ACC GTC GAA GAT GCC TTT ATG TCT TAT CTG ACA TCG TTT GAA CAA GTG    3264
Thr Val Glu Asp Ala Phe Met Ser Tyr Leu Thr Ser Phe Glu Gln Val
        1075                1080                1085

GCT AAT CTT AAA GTT ATT AGC GCA TAT CAC GAT AAT ATT AAT AAC GAT    3312
Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Ile Asn Asn Asp
        1090                1095                1100

CAA GGG CTG ACC TAT TTT ATC GGA CTC AGT GAA ACT GAT GCC GGT GAA    3360
Gln Gly Leu Thr Tyr Phe Ile Gly Leu Ser Glu Thr Asp Ala Gly Glu
    1105                1110                1115                1120

TAT TAT TGG CGC AGT GTC GAT CAC AGT AAA TTC AAC GAC GGT AAA TTC    3408
Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Phe Asn Asp Gly Lys Phe
                1125                1130                1135

GCG GCT AAT GCC TGG AGT GAA TGG CAT AAA ATT GAT TGT CCA ATT AAC    3456
Ala Ala Asn Ala Trp Ser Glu Trp His Lys Ile Asp Cys Pro Ile Asn
            1140                1145                1150

CCT TAT AAA AGC ACT ATC CGT CCA GTG ATA TAT AAA TCC CGC CTG TAT    3504
Pro Tyr Lys Ser Thr Ile Arg Pro Val Ile Tyr Lys Ser Arg Leu Tyr
        1155                1160                1165

CTG CTC TGG TTG GAA CAA AAG GAG ATC ACC AAA CAG ACA GGA AAT AGT    3552
Leu Leu Trp Leu Glu Gln Lys Glu Ile Thr Lys Gln Thr Gly Asn Ser
        1170                1175                1180

AAA GAT GGC TAT CAA ACT GAA ACG GAT TAT CGT TAT GAA CTA AAA TTG    3600
Lys Asp Gly Tyr Gln Thr Glu Thr Asp Tyr Arg Tyr Glu Leu Lys Leu
    1185                1190                1195                1200

GCG CAT ATC CGC TAT GAT GGC ACT TGG AAT ACG CCA ATC ACC TTT GAT    3648
Ala His Ile Arg Tyr Asp Gly Thr Trp Asn Thr Pro Ile Thr Phe Asp
                1205                1210                1215

GTC AAT AAA AAA ATA TCC GAG CTA AAA CTG GAA AAA AAT AGA GCG CCC    3696
Val Asn Lys Lys Ile Ser Glu Leu Lys Leu Glu Lys Asn Arg Ala Pro
                1220                1225                1230

GGA CTC TAT TGT GCC GGT TAT CAA GGT GAA GAT ACG TTG CTG GTG ATG    3744
Gly Leu Tyr Cys Ala Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met
        1235                1240                1245

TTT TAT AAC CAA CAA GAC ACA CTA GAT AGT TAT AAA AAC GCT TCA ATG    3792
Phe Tyr Asn Gln Gln Asp Thr Leu Asp Ser Tyr Lys Asn Ala Ser Met
        1250                1255                1260

CAA GGA CTA TAT ATC TTT GCT GAT ATG GCA TCC AAA GAT ATG ACC CCA    3840
Gln Gly Leu Tyr Ile Phe Ala Asp Met Ala Ser Lys Asp Met Thr Pro
    1265                1270                1275                1280

GAA CAG AGC AAT GTT TAT CGG GAT AAT AGC TAT CAA CAA TTT GAT ACC    3888
Glu Gln Ser Asn Val Tyr Arg Asp Asn Ser Tyr Gln Gln Phe Asp Thr
                1285                1290                1295

AAT AAT GTC AGA AGA GTG AAT AAC CGC TAT GCA GAG GAT TAT GAG ATT    3936
Asn Asn Val Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr Glu Ile
                1300                1305                1310

CCT TCC TCG GTA AGT AGC CGT AAA GAC TAT GGT TGG GGA GAT TAT TAC    3984
Pro Ser Ser Val Ser Ser Arg Lys Asp Tyr Gly Trp Gly Asp Tyr Tyr
                1315                1320                1325

CTC AGC ATG GTA TAT AAC GGA GAT ATT CCA ACT ATC AAT TAC AAA GCC    4032
Leu Ser Met Val Tyr Asn Gly Asp Ile Pro Thr Ile Asn Tyr Lys Ala
        1330                1335                1340

GCA TCA AGT GAT TTA AAA ATC TAT ATC TCA CCA AAA TTA AGA ATT ATT    4080
Ala Ser Ser Asp Leu Lys Ile Tyr Ile Ser Pro Lys Leu Arg Ile Ile
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              1345                1350              .1355              1360

      CAT AAT GGA TAT GAA GGA CAG AAG CGC AAT CAA TGC AAT CTG ATG AAT    4128
      His Asn Gly Tyr Glu Gly Gln Lys Arg Asn Gln Cys Asn Leu Met Asn
 5                            1365                1370              1375

      AAA TAT GGC AAA CTA GGT GAT AAA TTT ATT GTT TAT ACT AGC TTG GGG    4176
      Lys Tyr Gly Lys Leu Gly Asp Lys Phe Ile Val Tyr Thr Ser Leu Gly
                              1380                1385              1390
10
      GTC AAT CCA AAT AAC TCG TCA AAT AAG CTC ATG TTT TAC CCC GTC TAT    4224
      Val Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val Tyr
                              1395                1400              1405

15    CAA TAT AGC GGA AAC ACC AGT GGA CTC AAT CAA GGG AGA CTA CTA TTC    4272
      Gln Tyr Ser Gly Asn Thr Ser Gly Leu Asn Gln Gly Arg Leu Leu Phe
                    1410                1415                1420

      CAC CGT GAC ACC ACT TAT CCA TCT AAA GTA GAA GCT TGG ATT CCT GGA    4320
20    His Arg Asp Thr Thr Tyr Pro Ser Lys Val Glu Ala Trp Ile Pro Gly
      1425                1430                1435                1440

      GCA AAA CGT TCT CTA ACC AAC CAA AAT GCC GCC ATT GGT GAT GAT TAT    4368
      Ala Lys Arg Ser Leu Thr Asn Gln Asn Ala Ala Ile Gly Asp Asp Tyr
25                  1445                1450                1455

      GCT ACA GAC TCT CTG AAT AAA CCG GAT GAT CTT AAG CAA TAT ATC TTT    4416
      Ala Thr Asp Ser Leu Asn Lys Pro Asp Asp Leu Lys Gln Tyr Ile Phe
                    1460                1465                1470
30
      ATG ACT GAC AGT AAA GGG ACT GCT ACT GAT GTC TCA GGC CCA GTA GAG    4464
      Met Thr Asp Ser Lys Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu
                    1475                1480                1485

35    ATT AAT ACT GCA ATT TCT CCA GCA AAA GTT CAG ATA ATA GTC AAA GCG    4512
      Ile Asn Thr Ala Ile Ser Pro Ala Lys Val Gln Ile Ile Val Lys Ala
                    1490                1495                1500

      GGT GGC AAG GAG CAA ACT TTT ACC GCA GAT AAA GAT GTC TCC ATT CAG    4560
40    Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln
      1505                1510                1515                1520

      CCA TCA CCT AGC TTT GAT GAA ATG AAT TAT CAA TTT AAT GCC CTT GAA    4608
      Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu
45                  1525                1530                1535

      ATA GAC GGT TCT GGT CTG AAT TTT ATT AAC AAC TCA GCC AGT ATT GAT    4656
      Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp
                    1540                1545                1550
50
      GTT ACT TTT ACC GCA TTT GCG GAG GAT GGC CGC AAA CTG GGT TAT GAA    4704
      Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu
                    1555                1560                1565

55    AGT TTC AGT ATT CCT GTT ACC CTC AAG GTA AGT ACC GAT AAT GCC CTG    4752
      Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu
                    1570                1575                1580

      ACC CTG CAC CAT AAT GAA AAT GGT GCG CAA TAT ATG CAA TGG CAA TCC    4800
60    Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser
      1585                1590                1595                1600

      TAT CGT ACC CGC CTG AAT ACT CTA TTT GCC CGC CAG TTG GTT GCA CGC    4848
      Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg
65                  1605                1610                1615

      GCC ACC ACC GGA ATC GAT ACA ATT CTG AGT ATG GAA ACT CAG AAT ATT    4896
      Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile
                    1620                1625                1630
70
      CAG GAA CCG CAG TTA GGC AAA GGT TTC TAT GCT ACG TTC GTG ATA CCT    4944
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         Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro
             1635                1640                1645

  5      CCC TAT AAC CTA TCA ACT CAT GGT GAT GAA CGT TGG TTT AAG CTT TAT  4992
         Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr
             1650                1655                1660

         ATC AAA CAT GTT GTT GAT AAT AAT TCA CAT ATT ATC TAT TCA GGC CAG  5040
 10      Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln
         1665                1670                1675                1680

         CTA ACA GAT ACA AAT ATA AAC ATC ACA TTA TTT ATT CCT CTT GAT GAT  5088
         Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp
 15                          1685                1690                1695

         GTC CCA TTG AAT CAA GAT TAT CAC GCC AAG GTT TAT ATG ACC TTC AAG  5136
         Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys
                         1700                1705                1710

 20      AAA TCA CCA TCA GAT GGT ACC TGG TGG GGC CCT CAC TTT GTT AGA GAT  5184
         Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp
                 1715                1720                1725

         GAT AAA GGA ATA GTA ACA ATA AAC CCT AAA TCC ATT TTG ACC CAT TTT  5232
 25      Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe
             1730                1735                1740

         GAG AGC GTC AAT GTC CTG AAT AAT ATT AGT AGC GAA CCA ATG GAT TTC  5280
         Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe
 30      1745                1750                1755                1760

         AGC GGC GCT AAC AGC CTC TAT TTC TGG GAA CTG TTC TAC TAT ACC CCG  5328
         Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
                         1765                1770                1775
 35
         ATG CTG GTT GCT CAA CGT TTG CTG CAT GAA CAG AAC TTC GAT GAA GCC  5376
         Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala
                             1780                1785                1790

 40      AAC CGT TGG CTG AAA TAT GTC TGG AGT CCA TCC GGT TAT ATT GTC CAC  5424
         Asn Arg Trp Leu Lys Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val His
                         1795                1800                1805

         GGC CAG ATT CAG AAC TAC CAG TGG AAC GTC CGC CCG TTA CTG GAA GAC  5472
 45      Gly Gln Ile Gln Asn Tyr Gln Trp Asn Val Arg Pro Leu Leu Glu Asp
             1810                1815                1820

         ACC AGT TGG AAC AGT GAT CCT TTG GAT TCC GTC GAT CCT GAC GCG GTA  5520
         Thr Ser Trp Asn Ser Asp Pro Leu Asp Ser Val Asp Pro Asp Ala Val
 50      1825                1830                1835                1840

         GCA CAG CAC GAT CCA ATG CAC TAC AAA GTT TCA ACT TTT ATG CGT ACC  5568
         Ala Gln His Asp Pro Met His Tyr Lys Val Ser Thr Phe Met Arg Thr
                         1845                1850                1855
 55
         TTG GAT CTA TTG ATA GCA CGC GGC GAC CAT GCT TAT CGC CAA CTG GAA  5616
         Leu Asp Leu Leu Ile Ala Arg Gly Asp His Ala Tyr Arg Gln Leu Glu
                         1860                1865                1870

 60      CGA GAT ACA CTC AAC GAA GCG AAG ATG TGG TAT ATG CAA GCG CTG CAT  5664
         Arg Asp Thr Leu Asn Glu Ala Lys Met Trp Tyr Met Gln Ala Leu His
             1875                1880                1885

         CTA TTA GGT GAC AAA CCT TAT CTA CCG CTG AGT ACG ACA TGG AGT GAT  5712
 65      Leu Leu Gly Asp Lys Pro Tyr Leu Pro Leu Ser Thr Thr Trp Ser Asp
             1890                1895                1900

         CCA CGA CTA GAC AGA GCC GCG GAT ATC ACT ACC CAA AAT GCT CAC GAC  5760
         Pro Arg Leu Asp Arg Ala Ala Asp Ile Thr Thr Gln Asn Ala His Asp
 70      1905                1910                1915                1920
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     AGC GCA ATA GTC GCT CTG CGG CAG AAT ATA CCT ACA CCG GCA CCT TTA   5808
     Ser Ala Ile Val Ala Leu Arg Gln Asn Ile Pro Thr Pro Ala Pro Leu
                     1925                1930               1935
 5   TCA TTG CGC AGC GCT AAT ACC CTG ACT GAT CTC TTC CTG CCG CAA ATC   5856
     Ser Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile
                     1940                1945               1950
10   AAT GAA GTG ATG ATG AAT TAC TGG CAG ACA TTA GCT CAG AGA GTA TAC   5904
     Asn Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr
                     1955                1960               1965
     AAT CTG CGT CAT AAC CTC TCT ATC GAC GGC CAG CCG TTA TAT CTG CCA   5952
     Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro
15        1970                1975                1980
     ATC TAT GCC ACA CCG GCC GAT CCG AAA GCG TTA CTC AGC GCC GCC GTT   6000
     Ile Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val
     1985                1990                1995                2000
20
     GCC ACT TCT CAA GGT GGA GGC AAG CTA CCG GAA TCA TTT ATG TCC CTG   6048
     Ala Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu
                     2005                2010               2015
25   TGG CGT TTC CCG CAC ATG CTG GAA AAT GCG CGC GGC ATG GTT AGC CAG   6096
     Trp Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln
                     2020                2025               2030
     CTC ACC CAG TTC GGC TCC ACG TTA CAA AAT ATT ATC GAA CGT CAG GAC   6144
30   Leu Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp
                     2035                2040               2045
     GCG GAA GCG CTC AAT GCG TTA TTA CAA AAT CAG GCC GCC GAG CTG ATA   6192
     Ala Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile
35        2050                2055                2060
     TTG ACT AAC CTG AGC ATT CAG GAC AAA ACC ATT GAA GAA TTG GAT GCC   6240
     Leu Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala
     2065                2070                2075                2080
40
     GAG AAA ACG GTG TTG GAA AAA TCC AAA GCG GGA GCA CAA TCG CGC TTT   6288
     Glu Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe
                     2085                2090               2095
45   GAT AGC TAC GGC AAA CTG TAC GAT GAG AAT ATC AAC GCC GGT GAA AAC   6336
     Asp Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn
                     2100                2105               2110
     CAA GCC ATG ACG CTA CGA GCG TCC GCC GCC GGG CTT ACC ACG GCA GTT   6384
50   Gln Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val
                     2115                2120               2125
     CAG GCA TCC CGT CTG GCC GGT GCG GCG GCT GAT CTG GTG CCT AAC ATC   6432
     Gln Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile
55        2130                2135                2140
     TTC GGC TTT GCC GGT GGC GGC AGC CGT TGG GGG GCT ATC GCT GAG GCG   6480
     Phe Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala
     2145                2150                2155                2160
60
     ACA GGT TAT GTG ATG GAA TTC TCC GCG AAT GTT ATG AAC ACC GAA GCG   6528
     Thr Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala
                     2165                2170               2175
65   GAT AAA ATT AGC CAA TCT GAA ACC TAC CGT CGT CGC CGT CAG GAG TGG   6576
     Asp Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp
                     2180                2185               2190
     GAG ATC CAG CGG AAT AAT GCC GAA GCG GAA TTG AAG CAA ATC GAT GCT   6624
70   Glu Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala
                     2195                2200               2205
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     CAG CTC AAA TCA CTC GCT GTA CGC CGC GAA GCC GCC GTA TTG CAG AAA   6672
     Gln Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys
         2210              2215              2220
 5
     ACC AGT CTG AAA ACC CAA CAA GAA CAG ACC CAA TCT CAA TTG GCC TTC   6720
     Thr Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe
     2225              2230              2235              2240
10   CTG CAA CGT AAG TTC AGC AAT CAG GCG TTA TAC AAC TGG CTG CGT GGT   6768
     Leu Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly
                       2245              2250              2255
     CGA CTG GCG GCG ATT TAC TTC CAG TTC TAC GAT TTG GCC GTC GCG CGT   6816
15   Arg Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg
                   2260              2265              2270
     TGC CTG ATG GCA GAA CAA GCT TAC CGT TGG GAA CTC AAT GAT GAC TCT   6864
     Cys Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser
20           2275              2280              2285
     GCC CGC TTC ATT AAA CCG GGC GCC TGG CAG GGA ACC TAT GCC GGT CTG   6912
     Ala Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu
         2290              2295              2300
25   CTT GCA GGT GAA ACC TTG ATG CTG AGT CTG GCA CAA ATG GAA GAC GCT   6960
     Leu Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala
     2305              2310              2315              2320
30   CAT CTG AAA CGC GAT AAA CGC GCA TTA GAG GTT GAA CGC ACA GTA TCG   7008
     His Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser
                   2325              2330              2335
     CTG GCC GAA GTT TAT GCA GGA TTA CCA AAA GAT AAC GGT CCA TTT TCC   7056
35   Leu Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser
                   2340              2345              2350
     CTG GCT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC   7104
     Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala
40           2355              2360              2365
     GGC AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA   7152
     Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys
         2370              2375              2380
45   ACC TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA   7200
     Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu
     2385              2390              2395              2400
50   GAT TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC   7248
     Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser
                   2405              2410              2415
     GTC ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA   7296
55   Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile
                   2420              2425              2430
     TTG TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG   7344
     Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu
60           2435              2440              2445
     GCA GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC   7392
     Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe
         2450              2455              2460
65   AAC GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC   7440
     Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly
     2465              2470              2475              2480
70   ACG CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA   7488
     Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys
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                    2485                    2490                    2495
        CAA GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC  7536
        Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg
  5         2500             2505             2510

        TAC ACC ATT AAA TAA       7551
        Tyr Thr Ile Lys •••
                    2516
 10
```

(2) INFORMATION FOR SEQ ID NO:47:

         (i) SEQUENCE CHARACTERISTICS:
 15   (A) LENGTH: 2516 amino acids
              (B) TYPE: amino acids
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
 20
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47 (TcdA):
              Features   From   To   Description
              Peptide   1   2516   TcdA proteins
              Peptide   89   1937   TcdA$_{ii}$ peptide
 25   Fragment   89   100   TcdA$_{ii}$ N-terminus (SEQ ID NO:13)
              Fragment   284   299   (SEQ ID NO:38)
              Fragment   554   563   (SEQ ID NO:17)
              Fragment   1080   1092   (SEQ ID NO:23; 12/13)
              Fragment   1385   1400   (SEQ ID NO:18)
 30   Fragment   1478   1497   (SEQ ID NO:39)
              Fragment   1620   1642   (SEQ ID NO:21; 19/23)
              Fragment   1938   1948   (SEQ ID NO:41)
              Peptide   1938   2516   TcdA$_{iii}$ peptide
              Fragment   2327   2345   (SEQ ID NO:42)
 35   Fragment   2398   2408   (SEQ ID NO:43)

        Met Asn Glu Ser Val Lys Glu Ile Pro Asp Val Leu Lys Ser Gln Cys
          1   5   10   15

 40   Gly Phe Asn Cys Leu Thr Asp Ile Ser His Ser Ser Phe Asn Glu Phe
                      20   25   30

        Arg Gln Gln Val Ser Glu His Leu Ser Trp Ser Glu Thr His Asp Leu
                  35   40   45
 45
        Tyr His Asp Ala Gln Gln Ala Gln Lys Asp Asn Arg Leu Tyr Glu Ala
              50   55   60

        Arg Ile Leu Lys Arg Ala Asn Pro Gln Leu Gln Asn Ala Val His Leu
 50   65   70   75   80

        Ala Ile Leu Ala Pro Asn Ala Glu Leu Ile Gly Tyr Asn Asn Gln Phe
                          85   90   95

 55   Ser Gly Arg Ala Ser Gln Tyr Val Ala Pro Gly Thr Val Ser Ser Met
                          100   105   110

        Phe Ser Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Arg Asn
                  115   120   125
 60
        Leu His Ala Ser Asp Ser Val Tyr Tyr Leu Asp Thr Arg Arg Pro Asp
              130   135   140

        Leu Lys Ser Met Ala Leu Ser Gln Gln Asn Met Asp Ile Glu Leu Ser
 65   145   150   155   160

        Thr Leu Ser Leu Ser Asn Glu Leu Leu Leu Glu Ser Ile Lys Thr Glu
```

## SUBSTITUTE SHEET (RULE 26)

```
                              165                    170                    175
        Ser Lys Leu Glu Asn Tyr Thr Lys Val Met Glu Met Leu Ser Thr Phe
   5                180                185                190

        Arg Pro Ser Gly Ala Thr Pro Tyr His Asp Ala Tyr Glu Asn Val Arg
                    195                200                205

        Glu Val Ile Gln Leu Gln Asp Pro Gly Leu Glu Gln Leu Asn Ala Ser
  10        210                215                220

        Pro Ala Ile Ala Gly Leu Met His Gln Ala Ser Leu Leu Gly Ile Asn
        225                230                235                240

  15    Ala Ser Ile Ser Pro Glu Leu Phe Asn Ile Leu Thr Glu Glu Ile Thr
                        245                250                255

        Glu Gly Asn Ala Glu Glu Leu Tyr Lys Lys Asn Phe Gly Asn Ile Glu
                    260                265                270
  20
        Pro Ala Ser Leu Ala Met Pro Glu Tyr Leu Lys Arg Tyr Tyr Asn Leu
                    275                280                285

        Ser Asp Glu Glu Leu Ser Gln Phe Ile Gly Lys Ala Ser Asn Phe Gly
  25        290                295                300

        Gln Gln Glu Tyr Ser Asn Asn Gln Leu Ile Thr Pro Val Val Asn Ser
        305                310                315                320

  30    Ser Asp Gly Thr Val Lys Val Tyr Arg Ile Thr Arg Glu Tyr Thr Thr
                        325                330                335

        Asn Ala Tyr Gln Met Asp Val Glu Leu Phe Pro Phe Gly Gly Glu Asn
                    340                345                350
  35
        Tyr Arg Leu Asp Tyr Lys Phe Lys Asn Phe Tyr Asn Ala Ser Tyr Leu
                    355                360                365

        Ser Ile Lys Leu Asn Asp Lys Arg Glu Leu Val Arg Thr Glu Gly Ala
  40        370                375                380

        Pro Gln Val Asn Ile Glu Tyr Ser Ala Asn Ile Thr Leu Asn Thr Ala
        385                390                395                400

  45    Asp Ile Ser Gln Pro Phe Glu Ile Gly Leu Thr Arg Val Leu Pro Ser
                        405                410                415

        Gly Ser Trp Ala Tyr Ala Ala Ala Lys Phe Thr Val Glu Glu Tyr Asn
                    420                425                430
  50
        Gln Tyr Ser Phe Leu Leu Lys Leu Asn Lys Ala Ile Arg Leu Ser Arg
                    435                440                445

        Ala Thr Glu Leu Ser Pro Thr Ile Leu Glu Gly Ile Val Arg Ser Val
  55        450                455                460

        Asn Leu Gln Leu Asp Ile Asn Thr Asp Val Leu Gly Lys Val Phe Leu
        465                470                475                480

  60    Thr Lys Tyr Tyr Met Gln Arg Tyr Ala Ile His Ala Glu Thr Ala Leu
                        485                490                495

        Ile Leu Cys Asn Ala Pro Ile Ser Gln Arg Ser Tyr Asp Asn Gln Pro
                    500                505                510
  65
        Ser Gln Phe Asp Arg Leu Phe Asn Thr Pro Leu Leu Asn Gly Gln Tyr
                    515                520                525

        Phe Ser Thr Gly Asp Glu Glu Ile Asp Leu Asn Ser Gly Ser Thr Gly
  70        530                535                540
```

SUBSTITUTE SHEET (RULE 26)

```
        Asp Trp Arg Lys Thr Ile Leu Lys Arg Ala Phe Asn Ile Asp Asp Val
        545             550             555             560

 5      Ser Leu Phe Arg Leu Leu Lys Ile Thr Asp His Asp Asn Lys Asp Gly
                        565             570             575

        Lys Ile Lys Asn Asn Leu Lys Asn Leu Ser Asn Leu Tyr Ile Gly Lys
                        580             585             590

10      Leu Leu Ala Asp Ile His Gln Leu Thr Ile Asp Glu Leu Asp Leu Leu
                        595             600             605

        Leu Ile Ala Val Gly Glu Gly Lys Thr Asn Leu Ser Ala Ile Ser Asp
            610             615             620

15      Lys Gln Leu Ala Thr Leu Ile Arg Lys Leu Asn Thr Ile Thr Ser Trp
        625             630             635             640

        Leu His Thr Gln Lys Trp Ser Val Phe Gln Leu Phe Ile Met Thr Ser
20                      645             650             655

        Thr Ser Tyr Asn Lys Thr Leu Thr Pro Glu Ile Lys Asn Leu Leu Asp
                        660             665             670

25      Thr Val Tyr His Gly Leu Gln Gly Phe Asp Lys Asp Lys Ala Asp Leu
            675             680             685

        Leu His Val Met Ala Pro Tyr Ile Ala Ala Thr Leu Gln Leu Ser Ser
            690             695             700

30      Glu Asn Val Ala His Ser Val Leu Leu Trp Ala Asp Lys Leu Gln Pro
        705             710             715             720

        Gly Asp Gly Ala Met Thr Ala Glu Lys Phe Trp Asp Trp Leu Asn Thr
35                      725             730             735

        Lys Tyr Thr Pro Gly Ser Ser Glu Ala Val Glu Thr Gln Glu His Ile
                        740             745             750

40      Val Gln Tyr Cys Gln Ala Leu Ala Gln Leu Glu Met Val Tyr His Ser
            755             760             765

        Thr Gly Ile Asn Glu Asn Ala Phe Arg Leu Phe Val Thr Lys Pro Glu
            770             775             780

45      Met Phe Gly Ala Ala Thr Gly Ala Ala Pro Ala His Asp Ala Leu Ser
        785             790             795             800

        Leu Ile Met Leu Thr Arg Phe Ala Asp Trp Val Asn Ala Leu Gly Glu
50                      805             810             815

        Lys Ala Ser Ser Val Leu Ala Ala Phe Glu Ala Asn Ser Leu Thr Ala
                        820             825             830

55      Glu Gln Leu Ala Asp Ala Met Asn Leu Asp Ala Asn Leu Leu Leu Gln
                        835             840             845

        Ala Ser Ile Gln Ala Gln Asn His Gln His Leu Pro Pro Val Thr Pro
            850             855             860

60      Glu Asn Ala Phe Ser Cys Trp Thr Ser Ile Asn Thr Ile Leu Gln Trp
        865             870             875             880

        Val Asn Val Ala Gln Gln Leu Asn Val Ala Pro Gln Gly Val Ser Ala
65                      885             890             895

        Leu Val Gly Leu Asp Tyr Ile Gln Ser Met Lys Glu Thr Pro Thr Tyr
                        900             905             910

70      Ala Gln Trp Glu Asn Ala Ala Gly Val Leu Thr Ala Gly Leu Asn Ser
            915             920             925
```

SUBSTITUTE SHEET (RULE 26)

Gln Gln Ala Asn Thr Leu His Ala Phe Leu Asp Glu Ser Arg Ser Ala
    930   935   940

Ala Leu Ser Thr Tyr Tyr Ile Arg Gln Val Ala Lys Ala Ala Ala Ala
945   950   955   960

Ile Lys Ser Arg Asp Asp Leu Tyr Gln Tyr Leu Leu Ile Asp Asn Gln
            965   970   975

Val Ser Ala Ala Ile Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Ser
        980   985   990

Ile Gln Leu Tyr Val Asn Arg Ala Leu Glu Asn Val Glu Glu Asn Ala
    995   1000   1005

Asn Ser Gly Val Ile Ser Arg Gln Phe Phe Ile Asp Trp Asp Lys Tyr
    1010   1015   1020

Asn Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Gln Leu Val Tyr Tyr
    1025   1030   1035   1040

Pro Glu Asn Tyr Ile Asp Pro Thr Met Arg Ile Gly Gln Thr Lys Met
            1045   1050   1055

Met Asp Ala Leu Leu Gln Ser Val Ser Gln Ser Gln Leu Asn Ala Asp
        1060   1065   1070

Thr Val Glu Asp Ala Phe Met Ser Tyr Leu Thr Ser Phe Glu Gln Val
    1075   1080   1085

Ala Asn Leu Lys Val Ile Ser Ala Tyr His Asp Asn Ile Asn Asn Asp
    1090   1095   1100

Gln Gly Leu Thr Tyr Phe Ile Gly Leu Ser Glu Thr Asp Ala Gly Glu
1105   1110   1115   1120

Tyr Tyr Trp Arg Ser Val Asp His Ser Lys Phe Asn Asp Gly Lys Phe
            1125   1130   1135

Ala Ala Asn Ala Trp Ser Glu Trp His Lys Ile Asp Cys Pro Ile Asn
            1140   1145   1150

Pro Tyr Lys Ser Thr Ile Arg Pro Val Ile Tyr Lys Ser Arg Leu Tyr
    1155   1160   1165

Leu Leu Trp Leu Glu Gln Lys Glu Ile Thr Lys Gln Thr Gly Asn Ser
    1170   1175   1180

Lys Asp Gly Tyr Gln Thr Glu Thr Asp Tyr Arg Tyr Glu Leu Lys Leu
1185   1190   1195   1200

Ala His Ile Arg Tyr Asp Gly Thr Trp Asn Thr Pro Ile Thr Phe Asp
            1205   1210   1215

Val Asn Lys Lys Ile Ser Glu Leu Lys Leu Glu Lys Asn Arg Ala Pro
            1220   1225   1230

Gly Leu Tyr Cys Ala Gly Tyr Gln Gly Glu Asp Thr Leu Leu Val Met
        1235   1240   1245

Phe Tyr Asn Gln Gln Asp Thr Leu Asp Ser Tyr Lys Asn Ala Ser Met
    1250   1255   1260   ⎯

Gln Gly Leu Tyr Ile Phe Ala Asp Met Ala Ser Lys Asp Met Thr Pro
1265   1270   1275   1280

Glu Gln Ser Asn Val Tyr Arg Asp Asn Ser Tyr Gln Gln Phe Asp Thr
            1285   1290   1295

Asn Asn Val Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr Glu Ile

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SUBSTITUTE SHEET (RULE 26)

```
                   1300                  1305                  1310
       Pro Ser Ser Val Ser Ser Arg Lys Asp Tyr Gly Trp Gly Asp Tyr Tyr
           1315                  1320              1325
  5
       Leu Ser Met Val Tyr Asn Gly Asp Ile Pro Thr Ile Asn Tyr Lys Ala
           1330                  1335              1340

       Ala Ser Ser Asp Leu Lys Ile Tyr Ile Ser Pro Lys Leu Arg Ile Ile
 10    1345                  1350              1355                  1360

       His Asn Gly Tyr Glu Gly Gln Lys Arg Asn Gln Cys Asn Leu Met Asn
                   1365                  1370              1375

 15    Lys Tyr Gly Lys Leu Gly Asp Lys Phe Ile Val Tyr Thr Ser Leu Gly
                   1380                  1385              1390

       Val Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val Tyr
           1395                  1400              1405
 20
       Gln Tyr Ser Gly Asn Thr Ser Gly Leu Asn Gln Gly Arg Leu Leu Phe
           1410                  1415              1420

       His Arg Asp Thr Thr Tyr Pro Ser Lys Val Glu Ala Trp Ile Pro Gly
 25    1425                  1430              1435                  1440

       Ala Lys Arg Ser Leu Thr Asn Gln Asn Ala Ala Ile Gly Asp Asp Tyr
               1445                  1450              1455

 30    Ala Thr Asp Ser Leu Asn Lys Pro Asp Asp Leu Lys Gln Tyr Ile Phe
               1460                  1465              1470

       Met Thr Asp Ser Lys Gly Thr Ala Thr Asp Val Ser Gly Pro Val Glu
           1475                  1480              1485
 35
       Ile Asn Thr Ala Ile Ser Pro Ala Lys Val Gln Ile Ile Val Lys Ala
           1490                  1495              1500

       Gly Gly Lys Glu Gln Thr Phe Thr Ala Asp Lys Asp Val Ser Ile Gln
 40    1505                  1510              1515                  1520

       Pro Ser Pro Ser Phe Asp Glu Met Asn Tyr Gln Phe Asn Ala Leu Glu
               1525                  1530              1535

 45    Ile Asp Gly Ser Gly Leu Asn Phe Ile Asn Asn Ser Ala Ser Ile Asp
               1540                  1545              1550

       Val Thr Phe Thr Ala Phe Ala Glu Asp Gly Arg Lys Leu Gly Tyr Glu
           1555                  1560              1565
 50
       Ser Phe Ser Ile Pro Val Thr Leu Lys Val Ser Thr Asp Asn Ala Leu
           1570                  1575              1580

       Thr Leu His His Asn Glu Asn Gly Ala Gln Tyr Met Gln Trp Gln Ser
 55    1585                  1590              1595                  1600

       Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Ala Arg
                   1605                  1610              1615

 60    Ala Thr Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Asn Ile
               1620                  1625              1630

       Gln Glu Pro Gln Leu Gly Lys Gly Phe Tyr Ala Thr Phe Val Ile Pro
           1635                  1640              1645
 65
       Pro Tyr Asn Leu Ser Thr His Gly Asp Glu Arg Trp Phe Lys Leu Tyr
           1650                  1655              1660

       Ile Lys His Val Val Asp Asn Asn Ser His Ile Ile Tyr Ser Gly Gln
 70    1665                  1670              1675                  1680
```

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```
       Leu Thr Asp Thr Asn Ile Asn Ile Thr Leu Phe Ile Pro Leu Asp Asp
                     1685              1690              1695

  5    Val Pro Leu Asn Gln Asp Tyr His Ala Lys Val Tyr Met Thr Phe Lys
                     1700              1705              1710

       Lys Ser Pro Ser Asp Gly Thr Trp Trp Gly Pro His Phe Val Arg Asp
                     1715              1720              1725

 10    Asp Lys Gly Ile Val Thr Ile Asn Pro Lys Ser Ile Leu Thr His Phe
                 1730              1735              1740

       Glu Ser Val Asn Val Leu Asn Asn Ile Ser Ser Glu Pro Met Asp Phe
       1745              1750              1755              1760
 15
       Ser Gly Ala Asn Ser Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
                     1765              1770              1775

 20    Met Leu Val Ala Gln Arg Leu Leu His Glu Gln Asn Phe Asp Glu Ala
                     1780              1785              1790

       Asn Arg Trp Leu Lys Tyr Val Trp Ser Pro Ser Gly Tyr Ile Val His
                     1795              1800              1805

 25    Gly Gln Ile Gln Asn Tyr Gln Trp Asn Val Arg Pro Leu Leu Glu Asp
                     1810              1815              1820

       Thr Ser Trp Asn Ser Asp Pro Leu Asp Ser Val Asp Pro Asp Ala Val
       1825              1830              1835              1840
 30
       Ala Gln His Asp Pro Met His Tyr Lys Val Ser Thr Phe Met Arg Thr
                     1845              1850              1855

 35    Leu Asp Leu Leu Ile Ala Arg Gly Asp His Ala Tyr Arg Gln Leu Glu
                   1860              1865              1870

       Arg Asp Thr Leu Asn Glu Ala Lys Met Trp Tyr Met Gln Ala Leu His
                     1875              1880              1885

 40    Leu Leu Gly Asp Lys Pro Tyr Leu Pro Leu Ser Thr Thr Trp Ser Asp
                     1890              1895              1900

       Pro Arg Leu Asp Arg Ala Ala Asp Ile Thr Thr Gln Asn Ala His Asp
       1905              1910              1915              1920
 45
       Ser Ala Ile Val Ala Leu Arg Gln Asn Ile Pro Thr Pro Ala Pro Leu
                     1925              1930              1935

 50    Ser Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile
                   1940              1945              1950

       Asn Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr
                   1955              1960              1965

 55    Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro
                   1970              1975              1980

       Ile Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val
       1985              1990              1995              2000
 60
       Ala Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu
                     2005              2010              2015

 65    Trp Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln
                   2020              2025              2030

       Leu Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp
                   2035              2040              2045

 70    Ala Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile
                   2050              2055              2060
```

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```
        Leu Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala
        2065                2070            2075                2080

   5    Glu Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe
                        2085                2090                2095

        Asp Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn
                        2100                2105                2110
  10
        Gln Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val
                        2115                2120                2125

        Gln Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile
  15        2130                2135                2140

        Phe Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala
        2145                2150                2155                2160

  20    Thr Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala
                        2165                2170                2175

        Asp Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp
                        2180                2185                2190
  25
        Glu Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala
                2195                2200                2205

        Gln Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys
  30        2210                2215                2220

        Thr Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe
        2225                2230                2235                2240

  35    Leu Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly
                        2245                2250                2255

        Arg Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg
                        2260                2265                2270
  40
        Cys Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser
                2275                2280                2285

        Ala Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu
  45        2290                2295                2300

        Leu Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala
        2305                2310                2315                2320

  50    His Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser
                        2325                2330                2335

        Leu Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser
                        2340                2345                2350
  55
        Leu Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala
                2355                2360                2365

        Gly Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys
  60        2370                2375                2380

        Thr Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu
        2385                2390                2395                2400

  65    Asp Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser
                        2405                2410                2415

        Val Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile
                        2420                2425                2430
  70
        Leu Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu
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SUBSTITUTE SHEET (RULE 26)

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            2435                    2440          .          2445
                                                        _____
     Ala Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe
         2450                    2455            2460
  5
     Asn Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly
     2465                2470            2475            2480
 10  Thr Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys
                     2485            2490            2495
     Gln Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg
             2500            2505            2510
 15  Tyr Thr Ile Lys
             2516
```

```
 20  (2)  INFORMATION FOR SEQ ID NO:48:

         (i)  SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 5547 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: double
 25           (D) TOPOLOGY: linear
         (ii) MOLECULE TYPE: DNA (genomic)

         (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:48 (tcdAii coding
     region):
 30
     CTG ATA GGC TAT AAC AAT CAA TTT AGC GGT AGA GCC AGT CAA TAT GTT  48
     Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val
     1                   5                   10                  15
 35  GCG CCG GGT ACC GTT TCT TCC ATG TTC TCC CCC GCC GCT TAT TTG ACT  96
     Ala Pro Gly Thr Val Ser Ser Met Phe Ser Pro Ala Ala Tyr Leu Thr
                     20                  25                  30
     GAA CTT TAT CGT GAA GCA CGC AAT TTA CAC GCA AGT GAC TCC GTT TAT  144
 40  Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr
             35                  40                  45
     TAT CTG GAT ACC CGC CGC CCA GAT CTC AAA TCA ATG GCG CTC AGT CAG  192
     Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln
 45      50                  55                  60
     CAA AAT ATG GAT ATA GAA TTA TCC ACA CTC TCT TTG TCC AAT GAG CTG  240
     Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu
 50  65                  70                  75                  80
     TTA TTG GAA AGC ATT AAA ACT GAA TCT AAA CTG GAA AAC TAT ACT AAA  288
     Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys
                     85                  90                  95
 55  GTG ATG GAA ATG CTC TCC ACT TTC CGT CCT TCC GGC GCA ACG CCT TAT  336
     Val Met Glu Met Leu Ser Thr Phe Arg Pro Ser Gly Ala Thr Pro Tyr
                     100                 105                 110
     CAT GAT GCT TAT GAA AAT GTG CGT GAA GTT ATC CAG CTA CAA GAT CCT  384
 60  His Asp Ala Tyr Glu Asn Val Arg Glu Val Ile Gln Leu Gln Asp Pro
             115                 120                 125
     GGA CTT GAG CAA CTC AAT GCA TCA CCG GCA ATT GCC GGG TTG ATG CAT  432
     Gly Leu Glu Gln Leu Asn Ala Ser Pro Ala Ile Ala Gly Leu Met His
 65      130.                135                 140
     CAA GCC TCC CTA TTG GGT ATT AAC GCT TCA ATC TCG CCT GAG CTA TTT  480
     Gln Ala Ser Leu Leu Gly Ile Asn Ala Ser Ile Ser Pro Glu Leu Phe
     145                 150                 155                 160
```

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```
      AAT ATT CTG ACG GAG GAG ATT ACC GAA GGT AAT GCT GAG GAA CTT TAT 528
      Asn Ile Leu Thr Glu Glu Ile Thr Glu Gly Asn Ala Glu Glu Leu Tyr
 5                165                 170                 175

      AAG AAA AAT TTT GGT AAT ATC GAA CCG GCC TCA TTG GCT ATG CCG GAA 576
      Lys Lys Asn Phe Gly Asn Ile Glu Pro Ala Ser Leu Ala Met Pro Glu
                  180                 185                 190

10    TAC CTT AAA CGT TAT TAT AAT TTA AGC GAT GAA GAA CTT AGT CAG TTT 624
      Tyr Leu Lys Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe
                  195                 200                 205

      ATT GGT AAA GCC AGC AAT TTT GGT CAA CAG GAA TAT AGT AAT AAC CAA 672
15    Ile Gly Lys Ala Ser Asn Phe Gly Gln Gln Glu Tyr Ser Asn Asn Gln
                  210                 215                 220

      CTT ATT ACT CCG GTA GTC AAC AGC AGT GAT GGC ACG GTT AAG GTA TAT 720
      Leu Ile Thr Pro Val Val Asn Ser Ser Asp Gly Thr Val Lys Val Tyr
20    225                 230                 235                 240

      CGG ATC ACC CGC GAA TAT ACA ACC AAT GCT TAT CAA ATG GAT GTG GAG 768
      Arg Ile Thr Arg Glu Tyr Thr Thr Asn Ala Tyr Gln Met Asp Val Glu
                  245                 250                 255
25
      CTA TTT CCC TTC GGT GGT GAG AAT TAT CGG TTA GAT TAT AAA TTC AAA 816
      Leu Phe Pro Phe Gly Gly Glu Asn Tyr Arg Leu Asp Tyr Lys Phe Lys
                  260                 265                 270

30    AAT TTT TAT AAT GCC TCT TAT TTA TCC ATC AAG TTA AAT GAT AAA AGA 864
      Asn Phe Tyr Asn Ala Ser Tyr Leu Ser Ile Lys Leu Asn Asp Lys Arg
                  275                 280                 285

      GAA CTT GTT CGA ACT GAA GGC GCT CCT CAA GTC AAT ATA GAA TAC TCC 912
35    Glu Leu Val Arg Thr Glu Gly Ala Pro Gln Val Asn Ile Glu Tyr Ser
                  290                 295                 300

      GCA AAT ATC ACA TTA AAT ACC GCT GAT ATC AGT CAA CCT TTT GAA ATT 960
      Ala Asn Ile Thr Leu Asn Thr Ala Asp Ile Ser Gln Pro Phe Glu Ile
40    305                 310                 315                 320

      GGC CTG ACA CGA GTA CTT CCT TCC GGT TCT TGG GCA TAT GCC GCC GCA 1008
      Gly Leu Thr Arg Val Leu Pro Ser Gly Ser Trp Ala Tyr Ala Ala Ala
                  325                 330                 335
45
      AAA TTT ACC GTT GAA GAG TAT AAC CAA TAC TCT TTT CTG CTA AAA CTT 1056
      Lys Phe Thr Val Glu Glu Tyr Asn Gln Tyr Ser Phe Leu Leu Lys Leu
                  340                 345                 350

50    AAC AAG GCT ATT CGT CTA TCA CGT GCG ACA GAA TTG TCA CCC ACG ATT 1104
      Asn Lys Ala Ile Arg Leu Ser Arg Ala Thr Glu Leu Ser Pro Thr Ile
                  355                 360                 365

      CTG GAA GGC ATT GTG CGC AGT GTT AAT CTA CAA CTG GAT ATC AAC ACA 1152
55    Leu Glu Gly Ile Val Arg Ser Val Asn Leu Gln Leu Asp Ile Asn Thr
                  370                 375                 380

      GAC GTA TTA GGT AAA GTT TTT CTG ACT AAA TAT TAT ATG CAG CGT TAT 1200
      Asp Val Leu Gly Lys Val Phe Leu Thr Lys Tyr Tyr Met Gln Arg Tyr
60    385                 390                 395                 400

      GCT ATT CAT GCT GAA ACT GCC CTG ATA CTA TGC AAC GCG CCT ATT TCA 1248
      Ala Ile His Ala Glu Thr Ala Leu Ile Leu Cys Asn Ala Pro Ile Ser
                  405                 410                 415
65
      CAA CGT TCA TAT GAT AAT CAA CCT AGC CAA TTT GAT CGC CTG TTT AAT 1296
      Gln Arg Ser Tyr Asp Asn Gln Pro Ser Gln Phe Asp Arg Leu Phe Asn
                  420                 425                 430

70    ACG CCA TTA CTG AAC GGA CAA TAT TTT TCT ACC GGC GAT GAG GAG ATT 1344
      Thr Pro Leu Leu Asn Gly Gln Tyr Phe Ser Thr Gly Asp Glu Glu Ile
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SUBSTITUTE SHEET (RULE 26)

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                435                    440                    445

    GAT TTA AAT TCA GGT AGC ACC GGC GAT TGG CGA AAA ACC ATA CTT AAG 1392
    Asp Leu Asn Ser Gly Ser Thr Gly Asp Trp Arg Lys Thr Ile Leu Lys
5       450                455                460

    CGT GCA TTT AAT ATT GAT GAT GTC TCG CTC TTC CGC CTG CTT AAA ATT 1440
    Arg Ala Phe Asn Ile Asp Asp Val Ser Leu Phe Arg Leu Leu Lys Ile
    465                470                475                480
10
    ACC GAC CAT GAT AAT AAA GAT GGA AAA ATT AAA AAT AAC CTA AAG AAT 1488
    Thr Asp His Asp Asn Lys Asp Gly Lys Ile Lys Asn Asn Leu Lys Asn
                       485                490                495

15  CTT TCC AAT TTA TAT ATT GGA AAA TTA CTG GCA GAT ATT CAT CAA TTA 1536
    Leu Ser Asn Leu Tyr Ile Gly Lys Leu Leu Ala Asp Ile His Gln Leu
                   500                505                510

    ACC ATT GAT GAA CTG GAT TTA TTA CTG ATT GCC GTA GGT GAA GGA AAA 1584
20  Thr Ile Asp Glu Leu Asp Leu Leu Leu Ile Ala Val Gly Glu Gly Lys
                   515                520                525

    ACT AAT TTA TCC GCT ATC AGT GAT AAG CAA TTG GCT ACC CTG ATC AGA 1632
    Thr Asn Leu Ser Ala Ile Ser Asp Lys Gln Leu Ala Thr Leu Ile Arg
25      530                535                540

    AAA CTC AAT ACT ATT ACC AGC TGG CTA CAT ACA CAG AAG TGG AGT GTA 1680
    Lys Leu Asn Thr Ile Thr Ser Trp Leu His Thr Gln Lys Trp Ser Val
    545                550                555                560
30
    TTC CAG CTA TTT ATC ATG ACC TCC ACC AGC TAT AAC AAA ACG CTA ACG 1728
    Phe Gln Leu Phe Ile Met Thr Ser Thr Ser Tyr Asn Lys Thr Leu Thr
                       565                570                575

35  CCT GAA ATT AAG AAT TTG CTG GAT ACC GTC TAC CAC GGT TTA CAA GGT 1776
    Pro Glu Ile Lys Asn Leu Leu Asp Thr Val Tyr His Gly Leu Gln Gly
                   580                585                590

    TTT GAT AAA GAC AAA GCA GAT TTG CTA CAT GTC ATG GCG CCC TAT ATT 1824
40  Phe Asp Lys Asp Lys Ala Asp Leu Leu His Val Met Ala Pro Tyr Ile
                   595                600                605

    GCG GCC ACC TTG CAA TTA TCA TCG GAA AAT GTC GCC CAC TCG GTA CTC 1872
    Ala Ala Thr Leu Gln Leu Ser Ser Glu Asn Val Ala His Ser Val Leu
45      610                615                620

    CTT TGG GCA GAT AAG TTA CAG CCC GGC GAC GGC GCA ATG ACA GCA GAA 1920
    Leu Trp Ala Asp Lys Leu Gln Pro Gly Asp Gly Ala Met Thr Ala Glu
    625                630                635                640
50
    AAA TTC TGG GAC TGG TTG AAT ACT AAG TAT ACG CCG GGT TCA TCG GAA 1968
    Lys Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu
                       645                650                655

55  GCC GTA GAA ACG CAG GAA CAT ATC GTT CAG TAT TGT CAG GCT CTG GCA 2016
    Ala Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala
                   660                665                670

    CAA TTG GAA ATG GTT TAC CAT TCC ACC GGC ATC AAC GAA AAC GCC TTC 2064
60  Gln Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe
                   675                680                685

    CGT CTA TTT GTG ACA AAA CCA GAG ATG TTT GGC GCT GCA ACT GGA GCA 2112
    Arg Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala
65      690                695                700

    GCG CCC GCG CAT GAT GCC CTT TCA CTG ATT ATG CTG ACA CGT TTT GCG 2160
    Ala Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala
    705                710                715                720
70
    GAT TGG GTG AAC GCA CTA GGC GAA AAA GCG TCC TCG GTG CTA GCG GCA 2208
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SUBSTITUTE SHEET (RULE 26)

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        Asp Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala
                    725                 730         ___         735

  5     TTT GAA GCT AAC TCG TTA ACG GCA GAA CAA CTG GCT GAT GCC ATG AAT  2256
        Phe Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn
                        740                 745             750

        CTT GAT GCT AAT TTG CTG TTG CAA GCC AGT ATT CAA GCA CAA AAT CAT  2304
 10     Leu Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His
                    755                 760             765

        CAA CAT CTT CCC CCA GTA ACT CCA GAA AAT GCG TTC TCC TGT TGG ACA  2352
        Gln His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr
                770                 775             780
 15
        TCT ATC AAT ACT ATC CTG CAA TGG GTT AAT GTC GCA CAA CAA TTG AAT  2400
        Ser Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Asn
        785                 790             795             800

 20     GTC GCC CCA CAG GGC GTT TCC GCT TTG GTC GGG CTG GAT TAT ATT CAA  2448
        Val Ala Pro Gln Gly Val Ser Ala Leu Val Gly Leu Asp Tyr Ile Gln
                        805                 810             815

        TCA ATG AAA GAG ACA CCG ACC TAT GCC CAG TGG GAA AAC GCG GCA GGC  2496
 25     Ser Met Lys Glu Thr Pro Thr Tyr Ala Gln Trp Glu Asn Ala Ala Gly
                        820                 825             830

        GTA TTA ACC GCC GGG TTG AAT TCA CAA CAG GCT AAT ACA TTA CAC GCT  2544
 30     Val Leu Thr Ala Gly Leu Asn Ser Gln Gln Ala Asn Thr Leu His Ala
                        835                 840             845

        TTT CTG GAT GAA TCT CGC AGT GCC GCA TTA AGC ACC TAC TAT ATC CGT  2592
        Phe Leu Asp Glu Ser Arg Ser Ala Ala Leu Ser Thr Tyr Tyr Ile Arg
                    850                 855             860
 35
        CAA GTC GCC AAG GCA GCG GCG GCT ATT AAA AGC CGT GAT GAC TTG TAT  2640
        Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
        865                 870             875             880

 40     CAA TAC TTA CTG ATT GAT AAT CAG GTT TCT GCG GCA ATA AAA ACC ACC  2688
        Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
                        885                 890             895

        CGG ATC GCC GAA GCC ATT GCC AGT ATT CAA CTG TAC GTC AAC CGG GCA  2736
 45     Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
                        900                 905             910

        TTG GAA AAT GTG GAA GAA AAT GCC AAT TCG GGG GTT ATC AGC CGC CAA  2784
 50     Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
                915                 920             925

        TTC TTT ATC GAC TGG GAC AAA TAC AAT AAA CGC TAC AGC ACT TGG GCG  2832
        Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
                930                 935             940
 55
        GGT GTT TCT CAA TTA GTT TAC TAC CCG GAA AAC TAT ATT GAT CCG ACC  2880
        Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
        945                 950             955             960

 60     ATG CGT ATC GGA CAA ACC AAA ATG ATG GAC GCA TTA CTG CAA TCC GTC  2928
        Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
                        965                 970             975

        AGC CAA AGC CAA TTA AAC GCC GAT ACC GTC GAA GAT GCC TTT ATG TCT  2976
 65     Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
                        980                 985             990

        TAT CTG ACA TCG TTT GAA CAA GTG GCT AAT CTT AAA GTT ATT AGC GCA  3024
        Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala
 70             995                 1000            1005
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SUBSTITUTE SHEET (RULE 26)

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         TAT CAC GAT AAT ATT AAT AAC GAT CAA GGG CTG ACC TAT TTT ATC GGA 3072
         Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
             1010            1015                1020

    5    CTC AGT GAA ACT GAT GCC GGT GAA TAT TAT TGG CGC AGT GTC GAT CAC 3120
         Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
         1025            1030                1035                1040

   10    AGT AAA TTC AAC GAC GGT AAA TTC GCG GCT AAT GCC TGG AGT GAA TGG 3168
         Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
                         1045            1050                1055

         CAT AAA ATT GAT TGT CCA ATT AAC CCT TAT AAA AGC ACT ATC CGT CCA 3216
         His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
   15                1060            1065                1070

         GTG ATA TAT AAA TCC CGC CTG TAT CTG CTC TGG TTG GAA CAA AAG GAG 3264
         Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
                     1075            1080                1085
   20
         ATC ACC AAA CAG ACA GGA AAT AGT AAA GAT GGC TAT CAA ACT GAA ACG 3312
         Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
                     1090            1095                1100

   25    GAT TAT CGT TAT GAA CTA AAA TTG GCG CAT ATC CGC TAT GAT GGC ACT 3360
         Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
         1105            1110                1115                1120

         TGG AAT ACG CCA ATC ACC TTT GAT GTC AAT AAA AAA ATA TCC GAG CTA 3408
   30    Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
                         1125            1130                1135

         AAA CTG GAA AAA AAT AGA GCG CCC GGA CTC TAT TGT GCC GGT TAT CAA 3456
         Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
   35                1140            1145                1150

         GGT GAA GAT ACG TTG CTG GTG ATG TTT TAT AAC CAA CAA GAC ACA CTA 3504
         Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
                     1155            1160                1165
   40
         GAT AGT TAT AAA AAC GCT TCA ATG CAA GGA CTA TAT ATC TTT GCT GAT 3552
         Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
             1170            1175                1180

   45    ATG GCA TCC AAA GAT ATG ACC CCA GAA CAG AGC AAT GTT TAT CGG GAT 3600
         Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
         1185            1190                1195                1200

         AAT AGC TAT CAA CAA TTT GAT ACC AAT AAT GTC AGA AGA GTG AAT AAC 3648
   50    Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
                         1205            1210                1215

         CGC TAT GCA GAG GAT TAT GAG ATT CCT TCC TCG GTA AGT AGC CGT AAA 3696
         Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
   55                1220            1225                1230

         GAC TAT GGT TGG GGA GAT TAT TAC CTC AGC ATG GTA TAT AAC GGA GAT 3744
         Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp
                     1235            1240                1245
   60
         ATT CCA ACT ATC AAT TAC AAA GCC GCA TCA AGT GAT TTA AAA ATC TAT 3792
         Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
             1250            1255                1260

   65    ATC TCA CCA AAA TTA AGA ATT ATT CAT AAT GGA TAT GAA GGA CAG AAG 3840
         Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
         1265            1270                1275                1280

         CGC AAT CAA TGC AAT CTG ATG AAT AAA TAT GGC AAA CTA GGT GAT AAA 3888
   70    Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
                         1285            1290                1295
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SUBSTITUTE SHEET (RULE 26)

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     TTT ATT GTT TAT ACT AGC TTG GGG GTC AAT CCA AAT AAC TCG TCA AAT 3936
     Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
5                    1300                1305                1310

     AAG CTC ATG TTT TAC CCC GTC TAT CAA TAT AGC GGA AAC ACC AGT GGA 3984
     Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly
                     1315                1320                1325

10   CTC AAT CAA GGG AGA CTA CTA TTC CAC CGT GAC ACC ACT TAT CCA TCT 4032
     Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser
                 1330                1335                1340

     AAA GTA GAA GCT TGG ATT CCT GGA GCA AAA CGT TCT CTA ACC AAC CAA 4080
15   Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln
     1345                1350                1355                1360

     AAT GCC GCC ATT GGT GAT GAT TAT GCT ACA GAC TCT CTG AAT AAA CCG 4128
     Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro
20                   1365                1370                1375

     GAT GAT CTT AAG CAA TAT ATC TTT ATG ACT GAC AGT AAA GGG ACT GCT 4176
     Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala
                 1380                1385                1390
25
     ACT GAT GTC TCA GGC CCA GTA GAG ATT AAT ACT GCA ATT TCT CCA GCA 4224
     Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala
                 1395                1400                1405

30   AAA GTT CAG ATA ATA GTC AAA GCG GGT GGC AAG GAG CAA ACT TTT ACC 4272
     Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr
                 1410                1415                1420

     GCA GAT AAA GAT GTC TCC ATT CAG CCA TCA CCT AGC TTT GAT GAA ATG 4320
35   Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met
     1425                1430                1435                1440

     AAT TAT CAA TTT AAT GCC CTT GAA ATA GAC GGT TCT GGT CTG AAT TTT 4368
     Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe
40                   1445                1450                1455

     ATT AAC AAC TCA GCC AGT ATT GAT GTT ACT TTT ACC GCA TTT GCG GAG 4416
     Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu
                 1460                1465                1470
45
     GAT GGC CGC AAA CTG GGT TAT GAA AGT TTC AGT ATT CCT GTT ACC CTC 4464
     Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu
                 1475                1480                1485

50   AAG GTA AGT ACC GAT AAT GCC CTG ACC CTG CAC CAT AAT GAA AAT GGT 4512
     Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly
                 1490                1495                1500

     GCG CAA TAT ATG CAA TGG CAA TCC TAT CGT ACC CGC CTG AAT ACT CTA 4560
55   Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu
     1505                1510                1515                1520

     TTT GCC CGC CAG TTG GTT GCA CGC GCC ACC ACC GGA ATC GAT ACA ATT 4608
     Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile
60                   1525                1530                1535

     CTG AGT ATG GAA ACT CAG AAT ATT CAG GAA CCG CAG TTA GGC AAA GGT 4656
     Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly
                 1540                1545                1550
65
     TTC TAT GCT ACG TTC GTG ATA CCT CCC TAT AAC CTA TCA ACT CAT GGT 4704
     Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly
                 1555                1560                1565

70   GAT GAA CGT TGG TTT AAG CTT TAT ATC AAA CAT GTT GTT GAT AAT AAT 4752
     Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn
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SUBSTITUTE SHEET (RULE 26)

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            1570              1575              1580

    TCA CAT ATT ATC TAT TCA GGC CAG CTA ACA GAT ACA AAT ATA AAC ATC 4800
    Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile
 5  1585                  1590              1595              1600

    ACA TTA TTT ATT CCT CTT GAT GAT GTC CCA TTG AAT CAA GAT TAT CAC 4848
    Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His
                        1605              1610              1615
10
    GCC AAG GTT TAT ATG ACC TTC AAG AAA TCA CCA TCA GAT GGT ACC TGG 4896
    Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp
                    1620              1625              1630

15  TGG GGC CCT CAC TTT GTT AGA GAT GAT AAA GGA ATA GTA ACA ATA AAC 4944
    Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn
                1635              1640              1645

    CCT AAA TCC ATT TTG ACC CAT TTT GAG AGC GTC AAT GTC CTG AAT AAT 4992
20  Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn
                1650              1655              1660

    ATT AGT AGC GAA CCA ATG GAT TTC AGC GGC GCT AAC AGC CTC TAT TTC 5040
    Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe
25  1665                  1670              1675              1680

    TGG GAA CTG TTC TAC TAT ACC CCG ATG CTG GTT GCT CAA CGT TTG CTG 5088
    Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu
                        1685              1690              1695
30
    CAT GAA CAG AAC TTC GAT GAA GCC AAC CGT TGG CTG AAA TAT GTC TGG 5136
    His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp
                    1700              1705              1710

35  AGT CCA TCC GGT TAT ATT GTC CAC GGC CAG ATT CAG AAC TAC CAG TGG 5184
    Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp
                    1715              1720              1725

    AAC GTC CGC CCG TTA CTG GAA GAC ACC AGT TGG AAC AGT GAT CCT TTG 5232
40  Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu
                1730              1735              1740

    GAT TCC GTC GAT CCT GAC GCG GTA GCA CAG CAC GAT CCA ATG CAC TAC 5280
    Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr
45  1745                  1750              1755              1760

    AAA GTT TCA ACT TTT ATG CGT ACC TTG GAT CTA TTG ATA GCA CGC GGC 5328
    Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly
                        1765              1770              1775
50
    GAC CAT GCT TAT CGC CAA CTG GAA CGA GAT ACA CTC AAC GAA GCG AAG 5376
    Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys
                    1780              1785              1790

55  ATG TGG TAT ATG CAA GCG CTG CAT CTA TTA GGT GAC AAA CCT TAT CTA 5424
    Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu
                    1795              1800              1805

    CCG CTG AGT ACG ACA TGG AGT GAT CCA CGA CTA GAC AGA GCC GCG GAT 5472
60  Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp
                1810              1815              1820

    ATC ACT ACC CAA AAT GCT CAC GAC AGC GCA ATA GTC GCT CTG CGG CAG 5520
    Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln
65  1825              1830              1835              1840

    AAT ATA CCT ACA CCG GCA CCT TTA TCA      5547
    Asn Ile Pro Thr Pro Ala Pro Leu Ser
                    1845              1849
70
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SUBSTITUTE SHEET (RULE 26)

(2) INFORMATION FOR SEQ ID NO:49:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 1849 amino acids
      (B) TYPE: amino acids
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49 (TcdA$_{ii}$):

Features	From	To	Description
Peptide	1	1849	TcdA$_{ii}$ peptide
Fragment	1	12	TcdA$_{ii}$ N-terminus (SEQ ID NO:13)
Fragment	196	211	(SEQ ID NO:38)
Fragment	466	475	(SEQ ID NO:17)
Fragment	993	1004	(SEQ ID NO:23; 12/13)
Fragment	1297	1312	(SEQ ID NO:18)
Fragment	1390	1409	(SEQ ID NO:39)
Fragment	1532	1554	(SEQ ID NO:21; 19/23)

```
Leu Ile Gly Tyr Asn Asn Gln Phe Ser Gly Arg Ala Ser Gln Tyr Val
1               5                   10                    15

Ala Pro Gly Thr Val Ser Ser Met Phe Ser Pro Ala Ala Tyr Leu Thr
            20                  25                  30

Glu Leu Tyr Arg Glu Ala Arg Asn Leu His Ala Ser Asp Ser Val Tyr
        35                  40                  45

Tyr Leu Asp Thr Arg Arg Pro Asp Leu Lys Ser Met Ala Leu Ser Gln
    50                  55                  60

Gln Asn Met Asp Ile Glu Leu Ser Thr Leu Ser Leu Ser Asn Glu Leu
65                  70                  75                  80

Leu Leu Glu Ser Ile Lys Thr Glu Ser Lys Leu Glu Asn Tyr Thr Lys
                85                  90                  95

Val Met Glu Met Leu Ser Thr Phe Arg Pro Ser Gly Ala Thr Pro Tyr
                100                 105                 110

His Asp Ala Tyr Glu Asn Val Arg Glu Val Ile Gln Leu Gln Asp Pro
            115                 120                 125

Gly Leu Glu Gln Leu Asn Ala Ser Pro Ala Ile Ala Gly Leu Met His
    130                 135                 140

Gln Ala Ser Leu Leu Gly Ile Asn Ala Ser Ile Ser Pro Glu Leu Phe
145                 150                 155                 160

Asn Ile Leu Thr Glu Glu Ile Thr Glu Gly Asn Ala Glu Glu Leu Tyr
                165                 170                 175

Lys Lys Asn Phe Gly Asn Ile Glu Pro Ala Ser Leu Ala Met Pro Glu
            180                 185                 190

Tyr Leu Lys Arg Tyr Tyr Asn Leu Ser Asp Glu Glu Leu Ser Gln Phe
        195                 200                 205

Ile Gly Lys Ala Ser Asn Phe Gly Gln Gln Glu Tyr Ser Asn Asn Gln
    210                 215                 220

Leu Ile Thr Pro Val Val Asn Ser Ser Asp Gly Thr Val Lys Val Tyr
225                 230                 235                 240

Arg Ile Thr Arg Glu Tyr Thr Thr Asn Ala Tyr Gln Met Asp Val Glu
                245                 250                 255
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SUBSTITUTE SHEET (RULE 26)

	Leu	Phe	Pro 260	Phe	Gly	Gly	Glu	Asn	Tyr 265	Arg.	Leu	Asp	Tyr 270	Phe	Lys
5	Asn	Phe	Tyr 275	Asn	Ala	Ser	Tyr 280	Leu	Ser	Ile	Lys 285	Leu	Asn	Asp	Lys	Arg
	Glu	Leu 290	Val	Arg	Thr	Glu 295	Gly	Ala	Pro	Gln 300	Val	Asn	Ile	Glu	Tyr	Ser
10	Ala 305	Asn	Ile	Thr	Leu 310	Asn	Thr	Ala	Asp 315	Ile	Ser	Gln	Pro	Phe 320	Glu	Ile
15	Gly	Leu	Thr	Arg 325	Val	Leu	Pro	Ser	Gly 330	Ser	Trp	Ala	Tyr 335	Ala	Ala	Ala
	Lys	Phe	Thr	Val 340	Glu	Glu	Tyr	Asn 345	Gln	Tyr	Ser	Phe 350	Leu	Leu	Lys	Leu
20	Asn	Lys 355	Ala	Ile	Arg	Leu	Ser 360	Arg	Ala	Thr	Glu 365	Leu	Ser	Pro	Thr	Ile
	Leu	Glu 370	Gly	Ile	Val	Arg 375	Ser	Val	Asn	Leu 380	Gln	Leu	Asp	Ile	Asn	Thr
25	Asp 385	Val	Leu	Gly	Lys 390	Val	Phe	Leu	Thr 395	Lys	Tyr	Tyr	Met	Gln 400	Arg	Tyr
30	Ala	Ile	His	Ala	Glu 405	Thr	Ala	Leu	Ile 410	Leu	Cys	Asn	Ala	Pro 415	Ile	Ser
	Gln	Arg	Ser	Tyr 420	Asp	Asn	Gln	Pro	Ser 425	Gln	Phe	Asp	Arg 430	Leu	Phe	Asn
35	Thr	Pro	Leu 435	Leu	Asn	Gly	Gln	Tyr 440	Phe	Ser	Thr	Gly	Asp 445	Glu	Glu	Ile
	Asp	Leu 450	Asn	Ser	Gly	Ser	Thr 455	Gly	Asp	Trp	Arg	Lys 460	Thr	Ile	Leu	Lys
40	Arg 465	Ala	Phe	Asn	Ile	Asp 470	Asp	Val	Ser	Leu	Phe 475	Arg	Leu	Leu	Lys 480	Ile
45	Thr	Asp	His	Asp	Asn 485	Lys	Asp	Gly	Lys 490	Ile	Lys	Asn	Asn	Leu 495	Lys	Asn
	Leu	Ser	Asn	Leu 500	Tyr	Ile	Gly	Lys 505	Leu	Leu	Ala	Asp	Ile 510	His	Gln	Leu
50	Thr	Ile	Asp 515	Glu	Leu	Asp	Leu	Leu 520	Leu	Ile	Ala	Val	Gly 525	Glu	Gly	Lys
	Thr	Asn	Leu 530	Ser	Ala	Ile	Ser 535	Asp	Lys	Gln	Leu	Ala 540	Thr	Leu	Ile	Arg
55	Lys 545	Leu	Asn	Thr	Ile	Thr 550	Ser	Trp	Leu	His 555	Thr	Gln	Lys	Trp	Ser 560	Val
60	Phe	Gln	Leu	Phe	Ile 565	Met	Thr	Ser	Thr 570	Ser	Tyr	Asn	Lys	Thr 575	Leu	Thr
	Pro	Glu	Ile	Lys 580	Asn	Leu	Leu	Asp	Thr 585	Val	Tyr	His	Gly 590	Leu	Gln	Gly
65	Phe	Asp	Lys 595	Asp	Lys	Ala	Asp	Leu 600	Leu	His	Val	Met	Ala 605	Pro	Tyr	Ile
	Ala	Ala 610	Thr	Leu	Gln	Leu	Ser 615	Ser	Glu	Asn	Val 620	Ala	His	Ser	Val	Leu
70	Leu 625	Trp	Ala	Asp	Lys	Leu 630	Gln	Pro	Gly	Asp 635	Gly	Ala	Met	Thr	Ala 640	Glu

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```
      Lys Phe Trp Asp Trp Leu Asn Thr Lys Tyr Thr Pro Gly Ser Ser Glu
                  645             650             655

 5    Ala Val Glu Thr Gln Glu His Ile Val Gln Tyr Cys Gln Ala Leu Ala
                  660             665             670

      Gln Leu Glu Met Val Tyr His Ser Thr Gly Ile Asn Glu Asn Ala Phe
                  675             680             685
10
      Arg Leu Phe Val Thr Lys Pro Glu Met Phe Gly Ala Ala Thr Gly Ala
                  690             695             700

      Ala Pro Ala His Asp Ala Leu Ser Leu Ile Met Leu Thr Arg Phe Ala
15    705             710             715             720
      Asp Trp Val Asn Ala Leu Gly Glu Lys Ala Ser Ser Val Leu Ala Ala
                  725             730             735

      Phe Glu Ala Asn Ser Leu Thr Ala Glu Gln Leu Ala Asp Ala Met Asn
20                740             745             750

      Leu Asp Ala Asn Leu Leu Leu Gln Ala Ser Ile Gln Ala Gln Asn His
                  755             760             765

25    Gln His Leu Pro Pro Val Thr Pro Glu Asn Ala Phe Ser Cys Trp Thr
                  770             775             780

      Ser Ile Asn Thr Ile Leu Gln Trp Val Asn Val Ala Gln Gln Leu Asn
      785             790             795             800
30
      Val Ala Pro Gln Gly Val Ser Ala Leu Val Gly Leu Asp Tyr Ile Gln
                  805             810             815

      Ser Met Lys Glu Thr Pro Thr Tyr Ala Gln Trp Glu Asn Ala Ala Gly
35                820             825             830

      Val Leu Thr Ala Gly Leu Asn Ser Gln Gln Ala Asn Thr Leu His Ala
                  835             840             845

40    Phe Leu Asp Glu Ser Arg Ser Ala Ala Leu Ser Thr Tyr Tyr Ile Arg
                  850             855             860

      Gln Val Ala Lys Ala Ala Ala Ala Ile Lys Ser Arg Asp Asp Leu Tyr
      865             870             875             880
45
      Gln Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Ala Ile Lys Thr Thr
                  885             890             895

      Arg Ile Ala Glu Ala Ile Ala Ser Ile Gln Leu Tyr Val Asn Arg Ala
50                900             905             910

      Leu Glu Asn Val Glu Glu Asn Ala Asn Ser Gly Val Ile Ser Arg Gln
                  915             920             925

55    Phe Phe Ile Asp Trp Asp Lys Tyr Asn Lys Arg Tyr Ser Thr Trp Ala
                  930             935             940

      Gly Val Ser Gln Leu Val Tyr Tyr Pro Glu Asn Tyr Ile Asp Pro Thr
      945             950             955             960
60
      Met Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln Ser Val
                  965             970             975

      Ser Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe Met Ser
65                980             985             990

      Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile Ser Ala
                  995             1000            1005

70    Tyr His Asp Asn Ile Asn Asn Asp Gln Gly Leu Thr Tyr Phe Ile Gly
                  1010            1015            1020
```

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SUBSTITUTE SHEET (RULE 26)

```
Leu Ser Glu Thr Asp Ala Gly Glu Tyr Tyr Trp Arg Ser Val Asp His
1025                1030               1035                    1040

Ser Lys Phe Asn Asp Gly Lys Phe Ala Ala Asn Ala Trp Ser Glu Trp
               1045               1050               1055

His Lys Ile Asp Cys Pro Ile Asn Pro Tyr Lys Ser Thr Ile Arg Pro
           1060               1065               1070

Val Ile Tyr Lys Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln Lys Glu
        1075               1080               1085

Ile Thr Lys Gln Thr Gly Asn Ser Lys Asp Gly Tyr Gln Thr Glu Thr
       1090               1095               1100

Asp Tyr Arg Tyr Glu Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Thr
1105               1110               1115                    1120

Trp Asn Thr Pro Ile Thr Phe Asp Val Asn Lys Lys Ile Ser Glu Leu
               1125               1130               1135

Lys Leu Glu Lys Asn Arg Ala Pro Gly Leu Tyr Cys Ala Gly Tyr Gln
           1140               1145               1150

Gly Glu Asp Thr Leu Leu Val Met Phe Tyr Asn Gln Gln Asp Thr Leu
           1155               1160               1165

Asp Ser Tyr Lys Asn Ala Ser Met Gln Gly Leu Tyr Ile Phe Ala Asp
        1170               1175               1180

Met Ala Ser Lys Asp Met Thr Pro Glu Gln Ser Asn Val Tyr Arg Asp
1185               1190               1195                    1200

Asn Ser Tyr Gln Gln Phe Asp Thr Asn Asn Val Arg Arg Val Asn Asn
               1205               1210               1215

Arg Tyr Ala Glu Asp Tyr Glu Ile Pro Ser Ser Val Ser Ser Arg Lys
           1220               1225               1230

Asp Tyr Gly Trp Gly Asp Tyr Tyr Leu Ser Met Val Tyr Asn Gly Asp
        1235               1240               1245

Ile Pro Thr Ile Asn Tyr Lys Ala Ala Ser Ser Asp Leu Lys Ile Tyr
        1250               1255               1260

Ile Ser Pro Lys Leu Arg Ile Ile His Asn Gly Tyr Glu Gly Gln Lys
1265               1270               1275                    1280

Arg Asn Gln Cys Asn Leu Met Asn Lys Tyr Gly Lys Leu Gly Asp Lys
               1285               1290               1295

Phe Ile Val Tyr Thr Ser Leu Gly Val Asn Pro Asn Asn Ser Ser Asn
               1300               1305               1310

Lys Leu Met Phe Tyr Pro Val Tyr Gln Tyr Ser Gly Asn Thr Ser Gly
           1315               1320               1325

Leu Asn Gln Gly Arg Leu Leu Phe His Arg Asp Thr Thr Tyr Pro Ser
        1330               1335               1340

Lys Val Glu Ala Trp Ile Pro Gly Ala Lys Arg Ser Leu Thr Asn Gln
1345               1350               1355                    1360

Asn Ala Ala Ile Gly Asp Asp Tyr Ala Thr Asp Ser Leu Asn Lys Pro
           1365               1370               1375

Asp Asp Leu Lys Gln Tyr Ile Phe Met Thr Asp Ser Lys Gly Thr Ala
           1380               1385               1390

Thr Asp Val Ser Gly Pro Val Glu Ile Asn Thr Ala Ile Ser Pro Ala
```

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SUBSTITUTE SHEET (RULE 26)

```
                1395                1400          .        1405

        Lys Val Gln Ile Ile Val Lys Ala Gly Gly Lys Glu Gln Thr Phe Thr
            1410               1415            1420
  5
        Ala Asp Lys Asp Val Ser Ile Gln Pro Ser Pro Ser Phe Asp Glu Met
        1425               1430               1435                1440

        Asn Tyr Gln Phe Asn Ala Leu Glu Ile Asp Gly Ser Gly Leu Asn Phe
 10                     1445               1450                1455

        Ile Asn Asn Ser Ala Ser Ile Asp Val Thr Phe Thr Ala Phe Ala Glu
                    1460               1465                1470

 15     Asp Gly Arg Lys Leu Gly Tyr Glu Ser Phe Ser Ile Pro Val Thr Leu
                1475               1480               1485

        Lys Val Ser Thr Asp Asn Ala Leu Thr Leu His His Asn Glu Asn Gly
            1490               1495               1500
 20
        Ala Gln Tyr Met Gln Trp Gln Ser Tyr Arg Thr Arg Leu Asn Thr Leu
        1505               1510               1515                1520

        Phe Ala Arg Gln Leu Val Ala Arg Ala Thr Thr Gly Ile Asp Thr Ile
 25                     1525               1530                1535

        Leu Ser Met Glu Thr Gln Asn Ile Gln Glu Pro Gln Leu Gly Lys Gly
                1540               1545               1550

 30     Phe Tyr Ala Thr Phe Val Ile Pro Pro Tyr Asn Leu Ser Thr His Gly
                1555               1560               1565

        Asp Glu Arg Trp Phe Lys Leu Tyr Ile Lys His Val Val Asp Asn Asn
            1570               1575               1580
 35
        Ser His Ile Ile Tyr Ser Gly Gln Leu Thr Asp Thr Asn Ile Asn Ile
        1585               1590               1595                1600

        Thr Leu Phe Ile Pro Leu Asp Asp Val Pro Leu Asn Gln Asp Tyr His
 40                     1605               1610                1615

        Ala Lys Val Tyr Met Thr Phe Lys Lys Ser Pro Ser Asp Gly Thr Trp
                1620               1625               1630

 45     Trp Gly Pro His Phe Val Arg Asp Asp Lys Gly Ile Val Thr Ile Asn
                1635               1640               1645

        Pro Lys Ser Ile Leu Thr His Phe Glu Ser Val Asn Val Leu Asn Asn
            1650               1655               1660
 50
        Ile Ser Ser Glu Pro Met Asp Phe Ser Gly Ala Asn Ser Leu Tyr Phe
        1665               1670               1675                1680

        Trp Glu Leu Phe Tyr Tyr Thr Pro Met Leu Val Ala Gln Arg Leu Leu
 55                     1685               1690                1695

        His Glu Gln Asn Phe Asp Glu Ala Asn Arg Trp Leu Lys Tyr Val Trp
                1700               1705               1710

 60     Ser Pro Ser Gly Tyr Ile Val His Gly Gln Ile Gln Asn Tyr Gln Trp
                1715               1720               1725

        Asn Val Arg Pro Leu Leu Glu Asp Thr Ser Trp Asn Ser Asp Pro Leu
            1730               1735               1740
 65
        Asp Ser Val Asp Pro Asp Ala Val Ala Gln His Asp Pro Met His Tyr
        1745               1750               1755                1760

        Lys Val Ser Thr Phe Met Arg Thr Leu Asp Leu Leu Ile Ala Arg Gly
 70                     1765               1770                1775
```

SUBSTITUTE SHEET (RULE 26)

```
Asp His Ala Tyr Arg Gln Leu Glu Arg Asp Thr Leu Asn Glu Ala Lys
             1780                  1785              1790

Met Trp Tyr Met Gln Ala Leu His Leu Leu Gly Asp Lys Pro Tyr Leu
             1795                  1800              1805

Pro Leu Ser Thr Thr Trp Ser Asp Pro Arg Leu Asp Arg Ala Ala Asp
             1810                  1815              1820

Ile Thr Thr Gln Asn Ala His Asp Ser Ala Ile Val Ala Leu Arg Gln
1825                  1830                  1835              1840

Asn Ile Pro Thr Pro Ala Pro Leu Ser
             1845              1849
```

(2) INFORMATION FOR SEQ ID NO:50:

          (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 1740 base pairs
               (B) TYPE: nucleic acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear
          (ii) MOLECULE TYPE: DNA (genomic)

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50 (*tcdAiii* coding region):

```
TTG CGC AGC GCT AAT ACC CTG ACT GAT CTC TTC CTG CCG CAA ATC AAT 48
Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn
1                 5                   10                  15

GAA GTG ATG ATG AAT TAC TGG CAG ACA TTA GCT CAG AGA GTA TAC AAT 96
Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn
                 20                  25                  30

CTG CGT CAT AAC CTC TCT ATC GAC GGC CAG CCG TTA TAT CTG CCA ATC 144
Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro Ile
         35                  40                  45

TAT GCC ACA CCG GCC GAT CCG AAA GCG TTA CTC AGC GCC GCC GTT GCC 192
Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ala
         50                  55                  60

ACT TCT CAA GGT GGA GGC AAG CTA CCG GAA TCA TTT ATG TCC CTG TGG 240
Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu Trp
65                  70                  75                  80

CGT TTC CCG CAC ATG CTG GAA AAT GCG CGC GGC ATG GTT AGC CAG CTC 288
Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln Leu
                 85                  90                  95

ACC CAG TTC GGC TCC ACG TTA CAA AAT ATT ATC GAA CGT CAG GAC GCG 336
Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp Ala
                 100                 105                 110

GAA GCG CTC AAT GCG TTA TTA CAA AAT CAG GCC GCC GAG CTG ATA TTG 384
Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln Ala Ala Glu Leu Ile Leu
         115                 120                 125

ACT AAC CTG AGC ATT CAG GAC AAA ACC ATT GAA GAA TTG GAT GCC GAG 432
Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala Glu
         130                 135                 140

AAA ACG GTG TTG GAA AAA TCC AAA GCG GGA GCA CAA TCG CGC TTT GAT 480
Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe Asp
145                 150                 155                 160

AGC TAC GGC AAA CTG TAC GAT GAG AAT ATC AAC GCC GGT GAA AAC CAA 528
```

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```
          Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn Gln
                      165             170             175

   5      GCC ATG ACG CTA CGA GCG TCC GCC GCC GGG CTT ACC ACG GCA GTT CAG  576
          Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val Gln
                      180             185             190

          GCA TCC CGT CTG GCC GGT GCG GCG GCT GAT CTG GTG CCT AAC ATC TTC  624
  10      Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile Phe
                      195             200             205

          GGC TTT GCC GGT GGC GGC AGC CGT TGG GGG GCT ATC GCT GAG GCG ACA  672
          Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala Thr
  15          210             215             220

          GGT TAT GTG ATG GAA TTC TCC GCG AAT GTT ATG AAC ACC GAA GCG GAT  720
          Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala Asp
          225             230             235             240

  20      AAA ATT AGC CAA TCT GAA ACC TAC CGT CGT CGC CGT CAG GAG TGG GAG  768
          Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp Glu
                      245             250             255

  25      ATC CAG CGG AAT AAT GCC GAA GCG GAA TTG AAG CAA ATC GAT GCT CAG  816
          Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala Gln
                      260             265             270

          CTC AAA TCA CTC GCT GTA CGC CGC GAA GCC GCC GTA TTG CAG AAA ACC  864
          Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys Thr
  30          275             280             285

          AGT CTG AAA ACC CAA CAA GAA CAG ACC CAA TCT CAA TTG GCC TTC CTG  912
          Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe Leu
              290             295             300

  35
          CAA CGT AAG TTC AGC AAT CAG GCG TTA TAC AAC TGG CTG CGT GGT CGA  960
          Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly Arg
          305             310             315             320

  40      CTG GCG GCG ATT TAC TTC CAG TTC TAC GAT TTG GCC GTC GCG CGT TGC 1008
          Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys
                      325             330             335

          CTG ATG GCA GAA CAA GCT TAC CGT TGG GAA CTC AAT GAT GAC TCT GCC 1056
  45      Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala
                      340             345             350

          CGC TTC ATT AAA CCG GGC GCC TGG CAG GGA ACC TAT GCC GGT CTG CTT 1104
          Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
  50          355             360             365

          GCA GGT GAA ACC TTG ATG CTG AGT CTG GCA CAA ATG GAA GAC GCT CAT 1152
          Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His
              370             375             380

  55
          CTG AAA CGC GAT AAA CGC GCA TTA GAG GTT GAA CGC ACA GTA TCG CTG 1200
          Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
          385             390             395             400

  60      GCC GAA GTT TAT GCA GGA TTA CCA AAA GAT AAC GGT CCA TTT TCC CTG 1248
          Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu
                      405             410             415

          GCT CAG GAA ATT GAC AAG CTG GTG AGT CAA GGT TCA GGC AGT GCC GGC 1296
  65      Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly
                      420             425             430

          AGT GGT AAT AAT AAT TTG GCG TTC GGC GCC GGC ACG GAC ACT AAA ACC 1344
          Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr
  70          435             440             445
```

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SUBSTITUTE SHEET (RULE 26)

```
      TCT TTG CAG GCA TCA GTT TCA TTC GCT GAT TTG AAA ATT CGT GAA GAT 1392
      Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp
              450                 455                 460

 5    TAC CCG GCA TCG CTT GGC AAA ATT CGA CGT ATC AAA CAG ATC AGC GTC 1440
      Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val
      465                 470                 475                 480

      ACT TTG CCC GCG CTA CTG GGA CCG TAT CAG GAT GTA CAG GCA ATA TTG 1488
10    Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu
                          485                 490                 495

      TCT TAC GGC GAT AAA GCC GGA TTA GCT AAC GGC TGT GAA GCG CTG GCA 1536
      Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala
15                500                 505                 510

      GTT TCT CAC GGT ATG AAT GAC AGC GGC CAA TTC CAG CTC GAT TTC AAC 1584
      Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn
              515                 520                 525
20
      GAT GGC AAA TTC CTG CCA TTC GAA GGC ATC GCC ATT GAT CAA GGC ACG 1632
      Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr
              530                 535                 540

25    CTG ACA CTG AGC TTC CCA AAT GCA TCT ATG CCG GAG AAA GGT AAA CAA 1680
      Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln
      545                 550                 555                 560

      GCC ACT ATG TTA AAA ACC CTG AAC GAT ATC ATT TTG CAT ATT CGC TAC 1728
30    Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr
                          565                 570                 575

      ACC ATT AAA TAA    1740
      Thr Ile Lys •••
35            579
```

(2) INFORMATION FOR SEQ ID NO:51:

40   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 579 amino acids
                (B) TYPE: amino acids
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
45   (ii) MOLECULE TYPE: protein

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51 (TcdA$_{iii}$):

```
      Leu Arg Ser Ala Asn Thr Leu Thr Asp Leu Phe Leu Pro Gln Ile Asn
50     1               5                  10                  15

      Glu Val Met Met Asn Tyr Trp Gln Thr Leu Ala Gln Arg Val Tyr Asn
                      20                  25                  30

55    Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Tyr Leu Pro Ile
                  35                  40                  45

      Tyr Ala Thr Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ala
              50                  55                  60
60
      Thr Ser Gln Gly Gly Gly Lys Leu Pro Glu Ser Phe Met Ser Leu Trp
      65                  70                  75                  80

      Arg Phe Pro His Met Leu Glu Asn Ala Arg Gly Met Val Ser Gln Leu
65                    85                  90                  95

      Thr Gln Phe Gly Ser Thr Leu Gln Asn Ile Ile Glu Arg Gln Asp Ala
                      100                 105                 110
```

```
       Glu Ala Leu Asn Ala Leu Leu Gln Asn Gln·Ala Ala Glu Leu Ile Leu
               115             120             125
   5   Thr Asn Leu Ser Ile Gln Asp Lys Thr Ile Glu Glu Leu Asp Ala Glu
               130             135             140
       Lys Thr Val Leu Glu Lys Ser Lys Ala Gly Ala Gln Ser Arg Phe Asp
       145             150             155             160
  10   Ser Tyr Gly Lys Leu Tyr Asp Glu Asn Ile Asn Ala Gly Glu Asn Gln
                   165             170             175
       Ala Met Thr Leu Arg Ala Ser Ala Ala Gly Leu Thr Thr Ala Val Gln
                   180             185             190
  15   Ala Ser Arg Leu Ala Gly Ala Ala Ala Asp Leu Val Pro Asn Ile Phe
                   195             200             205
       Gly Phe Ala Gly Gly Gly Ser Arg Trp Gly Ala Ile Ala Glu Ala Thr
  20       210             215             220
       Gly Tyr Val Met Glu Phe Ser Ala Asn Val Met Asn Thr Glu Ala Asp
       225             230             235             240
  25   Lys Ile Ser Gln Ser Glu Thr Tyr Arg Arg Arg Arg Gln Glu Trp Glu
                   245             250             255
       Ile Gln Arg Asn Asn Ala Glu Ala Glu Leu Lys Gln Ile Asp Ala Gln
                   260             265             270
  30   Leu Lys Ser Leu Ala Val Arg Arg Glu Ala Ala Val Leu Gln Lys Thr
                   275             280             285
       Ser Leu Lys Thr Gln Gln Glu Gln Thr Gln Ser Gln Leu Ala Phe Leu
  35       290             295             300
       Gln Arg Lys Phe Ser Asn Gln Ala Leu Tyr Asn Trp Leu Arg Gly Arg
       305             310             315             320
  40   Leu Ala Ala Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ala Arg Cys
                   325             330             335
       Leu Met Ala Glu Gln Ala Tyr Arg Trp Glu Leu Asn Asp Asp Ser Ala
                   340             345             350
  45   Arg Phe Ile Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
                   355             360             365
       Ala Gly Glu Thr Leu Met Leu Ser Leu Ala Gln Met Glu Asp Ala His
  50       370             375             380
       Leu Lys Arg Asp Lys Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
       385             390             395             400
  55   Ala Glu Val Tyr Ala Gly Leu Pro Lys Asp Asn Gly Pro Phe Ser Leu
                   405             410             415
       Ala Gln Glu Ile Asp Lys Leu Val Ser Gln Gly Ser Gly Ser Ala Gly
  60       420             425             430
       Ser Gly Asn Asn Asn Leu Ala Phe Gly Ala Gly Thr Asp Thr Lys Thr
                   435             440             445
  65   Ser Leu Gln Ala Ser Val Ser Phe Ala Asp Leu Lys Ile Arg Glu Asp
               450             455             460
       Tyr Pro Ala Ser Leu Gly Lys Ile Arg Arg Ile Lys Gln Ile Ser Val
       465             470             475             480
  70   Thr Leu Pro Ala Leu Leu Gly Pro Tyr Gln Asp Val Gln Ala Ile Leu
                   485             490             495
```

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SUBSTITUTE SHEET (RULE 26)

```
      Ser Tyr Gly Asp Lys Ala Gly Leu Ala Asn Gly Cys Glu Ala Leu Ala
                  500             505             510

 5    Val Ser His Gly Met Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn
                  515             520             525

      Asp Gly Lys Phe Leu Pro Phe Glu Gly Ile Ala Ile Asp Gln Gly Thr
                  530             535             540
10
      Leu Thr Leu Ser Phe Pro Asn Ala Ser Met Pro Glu Lys Gly Lys Gln
      545             550             555             560

      Ala Thr Met Leu Lys Thr Leu Asn Asp Ile Ile Leu His Ile Arg Tyr
15                    565             570             575

      Thr Ile Lys •••
                  579

20
      (2) INFORMATION FOR SEQ ID NO:52:

             (i) SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 5532 base pairs
25               (B) TYPE: nucleic acid
                 (C) STRANDEDNESS: double
                 (D) TOPOLOGY: linear
             (ii) MOLECULE TYPE: DNA (genomic)

30           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52 (tcbAii coding
      region):

      TTT ATA CAA GGT TAT AGT GAT CTG TTT GGT AAT CGT GCT GAT AAC TAT 48
      Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr
35    1               5               10              15

      GCC GCG CCG GGC TCG GTT GCA TCG ATG TTC TCA CCG GCG GCT TAT TTG 96
      Ala Ala Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala Tyr Leu
                  20              25              30
40
      ACG GAA TTG TAC CGT GAA GCC AAA AAC TTG CAT GAC AGC AGC TCA ATT 144
      Thr Glu Leu Tyr Arg Glu Ala Lys Asn Leu His Asp Ser Ser Ser Ile
                  35              40              45

45    TAT TAC CTA GAT AAA CGT CGC CCG GAT TTA GCA AGC TTA ATG CTC AGC 192
      Tyr Tyr Leu Asp Lys Arg Arg Pro Asp Leu Ala Ser Leu Met Leu Ser
                  50              55              60

      CAG AAA AAT ATG GAT GAG GAA ATT TCA ACG CTG GCT CTC TCT AAT GAA 240
      Gln Lys Asn Met Asp Glu Glu Ile Ser Thr Leu Ala Leu Ser Asn Glu
50    65              70              75              80

      TTG TGC CTT GCC GGG ATC GAA ACA AAA ACA GGA AAA TCA CAA GAT GAA 288
      Leu Cys Leu Ala Gly Ile Glu Thr Lys Thr Gly Lys Ser Gln Asp Glu
55                    85              90              95

      GTG ATG GAT ATG TTG TCA ACT TAT CGT TTA AGT GGA GAG ACA CCT TAT 336
      Val Met Asp Met Leu Ser Thr Tyr Arg Leu Ser Gly Glu Thr Pro Tyr
                  100             105             110
60
      CAT CAC GCT TAT GAA ACT GTT CGT GAA ATC GTT CAT GAA CGT GAT CCA 384
      His His Ala Tyr Glu Thr Val Arg Glu Ile Val His Glu Arg Asp Pro
                  115             120             125

65    GGA TTT CGT CAT TTG TCA CAG GCA CCC ATT GTT GCT GCT AAG CTC GAT 432
      Gly Phe Arg His Leu Ser Gln Ala Pro Ile Val Ala Ala Lys Leu Asp
                  130             135             140

      CCT GTG ACT TTG TTG GGT ATT AGC TCC CAT ATT TCG CCA GAA CTG TAT 480
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SUBSTITUTE SHEET (RULE 26)

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           Pro Val Thr Leu Leu Gly Ile Ser Ser His Ile Ser Pro Glu Leu Tyr
           145                 150              ·155         Pro Glu     160

        5  AAC TTG CTG ATT GAG GAG ATC CCG GAA AAA GAT GAA GCC GCG CTT GAT 528
           Asn Leu Leu Ile Glu Glu Ile Pro Glu Lys Asp Glu Ala Ala Leu Asp
                           165              170              175

           ACG CTT TAT AAA ACA AAC TTT GGC GAT ATT ACT ACT GCT CAG TTA ATG 576
       10  Thr Leu Tyr Lys Thr Asn Phe Gly Asp Ile Thr Thr Ala Gln Leu Met
                       180              185              190

           TCC CCA AGT TAT CTG GCC CGG TAT TAT GGC GTC TCA CCG GAA GAT ATT 624
           Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr Gly Val Ser Pro Glu Asp Ile
                   195              200              205

       15  GCC TAC GTG ACG ACT TCA TTA TCA CAT GTT GGA TAT AGC AGT GAT ATT 672
           Ala Tyr Val Thr Thr Ser Leu Ser His Val Gly Tyr Ser Ser Asp Ile
               210              215              220

       20  CTG GTT ATT CCG TTG GTC GAT GGT GTG GGT AAG ATG GAA GTA GTT CGT 720
           Leu Val Ile Pro Leu Val Asp Gly Val Gly Lys Met Glu Val Val Arg
           225              230              235              240

       25  GTT ACC CGA ACA CCA TCG GAT AAT TAT ACC AGT CAG ACG AAT TAT ATT 768
           Val Thr Arg Thr Pro Ser Asp Asn Tyr Thr Ser Gln Thr Asn Tyr Ile
                           245              250              255

           GAG CTG TAT CCA CAG GGT GGC GAC AAT TAT TTG ATC AAA TAC AAT CTA 816
           Glu Leu Tyr Pro Gln Gly Gly Asp Asn Tyr Leu Ile Lys Tyr Asn Leu
       30                  260              265              270

           AGC AAT AGT TTT GGT TTG GAT GAT TTT TAT CTG CAA TAT AAA GAT GGT 864
           Ser Asn Ser Phe Gly Leu Asp Asp Phe Tyr Leu Gln Tyr Lys Asp Gly
                       275              280              285

       35  TCC GCT GAT TGG ACT GAG ATT GCC CAT AAT CCC TAT CCT GAT ATG GTC 912
           Ser Ala Asp Trp Thr Glu Ile Ala His Asn Pro Tyr Pro Asp Met Val
                   290              295              300

       40  ATA AAT CAA AAG TAT GAA TCA CAG GCG ACA ATC AAA CGT AGT GAC TCT 960
           Ile Asn Gln Lys Tyr Glu Ser Gln Ala Thr Ile Lys Arg Ser Asp Ser
           305              310              315              320

       45  GAC AAT ATA CTC AGT ATA GGG TTA CAA AGA TGG CAT AGC GGT AGT TAT 1008
           Asp Asn Ile Leu Ser Ile Gly Leu Gln Arg Trp His Ser Gly Ser Tyr
                           325              330              335

           AAT TTT GCC GCC GCC AAT TTT AAA ATT GAC CAA TAC TCC CCG AAA GCT 1056
           Asn Phe Ala Ala Ala Asn Phe Lys Ile Asp Gln Tyr Ser Pro Lys Ala
       50                  340              345              350

           TTC CTG CTT AAA ATG AAT AAG GCT ATT CGG TTG CTC AAA GCT ACC GGC 1104
           Phe Leu Leu Lys Met Asn Lys Ala Ile Arg Leu Leu Lys Ala Thr Gly
                       355              360              365

       55  CTC TCT TTT GCT ACG TTG GAG CGT ATT GTT GAT AGT GTT AAT AGC ACC 1152
           Leu Ser Phe Ala Thr Leu Glu Arg Ile Val Asp Ser Val Asn Ser Thr
                   370              375              380

       60  AAA TCC ATC ACG GTT GAG GTA TTA AAC AAG GTT TAT CGG GTA AAA TTC 1200
           Lys Ser Ile Thr Val Glu Val Leu Asn Lys Val Tyr Arg Val Lys Phe
           385              390              395              400

       65  TAT ATT GAT CGT TAT GGC ATC AGT GAA GAG ACA GCC GCT ATT TTG GCT 1248
           Tyr Ile Asp Arg Tyr Gly Ile Ser Glu Glu Thr Ala Ala Ile Leu Ala
                           405              410              415

           AAT ATT AAT ATC TCT CAG CAA GCT GTT GGC AAT CAG CTT AGC CAG TTT 1296
           Asn Ile Asn Ile Ser Gln Gln Ala Val Gly Asn Gln Leu Ser Gln Phe
       70                  420              425              430
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      GAG CAA CTA TTT AAT CAC CCG CCG CTC AAT GGT ATT CGC TAT GAA ATC 1344
      Glu Gln Leu Phe Asn His Pro Pro Leu Asn Gly Ile Arg Tyr Glu Ile
              435             440                 445

 5    AGT GAG GAC AAC TCC AAA CAT CTT CCT AAT CCT GAT CTG AAC CTT AAA 1392
      Ser Glu Asp Asn Ser Lys His Leu Pro Asn Pro Asp Leu Asn Leu Lys
              450             455                 460

10    CCA GAC AGT ACC GGT GAT GAT CAA CGC AAG GCG GTT TTA AAA CGC GCG 1440
      Pro Asp Ser Thr Gly Asp Asp Gln Arg Lys Ala Val Leu Lys Arg Ala
      465             470                 475                 480

      TTT CAG GTT AAC GCC AGT GAG TTG TAT CAG ATG TTA TTG ATC ACT GAT 1488
      Phe Gln Val Asn Ala Ser Glu Leu Tyr Gln Met Leu Leu Ile Thr Asp
15                  485                 490                 495

      CGT AAA GAA GAC GGT GTT ATC AAA AAT AAC TTA GAG AAT TTG TCT GAT 1536
      Arg Lys Glu Asp Gly Val Ile Lys Asn Asn Leu Glu Asn Leu Ser Asp
                  500                 505                 510
20
      CTG TAT TTG GTT AGT TTG CTG GCC CAG ATT CAT AAC CTG ACT ATT GCT 1584
      Leu Tyr Leu Val Ser Leu Leu Ala Gln Ile His Asn Leu Thr Ile Ala
                  515                 520                 525

25    GAA TTG AAC ATT TTG TTG GTG ATT TGT GGC TAT GGC GAC ACC AAC ATT 1632
      Glu Leu Asn Ile Leu Leu Val Ile Cys Gly Tyr Gly Asp Thr Asn Ile
              530                 535                 540

      TAT CAG ATT ACC GAC GAT AAT TTA GCC AAA ATA GTG GAA ACA TTG TTG 1680
30    Tyr Gln Ile Thr Asp Asp Asn Leu Ala Lys Ile Val Glu Thr Leu Leu
      545             550                 555                 560

      TGG ATC ACT CAA TGG TTG AAG ACC CAA AAA TGG ACA GTT ACC GAC CTG 1728
      Trp Ile Thr Gln Trp Leu Lys Thr Gln Lys Trp Thr Val Thr Asp Leu
35                  565                 570                 575

      TTT CTG ATG ACC ACG GCC ACT TAC AGC ACC ACT TTA ACG CCA GAA ATT 1776
      Phe Leu Met Thr Thr Ala Thr Tyr Ser Thr Thr Leu Thr Pro Glu Ile
                  580                 585                 590
40
      AGC AAT CTG ACG GCT ACG TTG TCT TCA ACT TTG CAT GGC AAA GAG AGT 1824
      Ser Asn Leu Thr Ala Thr Leu Ser Ser Thr Leu His Gly Lys Glu Ser
                  595                 600                 605

45    CTG ATT GGG GAA GAT CTG AAA AGA GCA ATG GCG CCT TGC TTC ACT TCG 1872
      Leu Ile Gly Glu Asp Leu Lys Arg Ala Met Ala Pro Cys Phe Thr Ser
              610                 615                 620

      GCT TTG CAT TTG ACT TCT CAA GAA GTT GCG TAT GAC CTG CTG TTG TGG 1920
50    Ala Leu His Leu Thr Ser Gln Glu Val Ala Tyr Asp Leu Leu Leu Trp
      625             630                 635                 640

      ATA GAC CAG ATT CAA CCG GCA CAA ATA ACT GTT GAT GGG TTT TGG GAA 1968
      Ile Asp Gln Ile Gln Pro Ala Gln Ile Thr Val Asp Gly Phe Trp Glu
55                  645                 650                 655

      GAA GTG CAA ACA ACA CCA ACC AGC TTG AAG GTG ATT ACC TTT GCT CAG 2016
      Glu Val Gln Thr Thr Pro Thr Ser Leu Lys Val Ile Thr Phe Ala Gln
                  660                 665                 670
60
      GTG CTG GCA CAA TTG AGC CTG ATC TAT CGT CGT ATT GGG TTA AGT GAA 2064
      Val Leu Ala Gln Leu Ser Leu Ile Tyr Arg Arg Ile Gly Leu Ser Glu
                  675                 680                 685

65    ACG GAA CTG TCA CTG ATC GTG ACT CAA TCT TCT CTG CTA GTG GCA GGC 2112
      Thr Glu Leu Ser Leu Ile Val Thr Gln Ser Ser Leu Leu Val Ala Gly
              690                 695                 700

      AAA AGC ATA CTG GAT CAC GGT CTG TTA ACC CTG ATG GCC TTG GAA GGT 2160
70    Lys Ser Ile Leu Asp His Gly Leu Leu Thr Leu Met Ala Leu Glu Gly
      705             710                 715                 720
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SUBSTITUTE SHEET (RULE 26)

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     TTT CAT ACC TGG GTT AAT GGC TTG GGG CAA CAT GCC TCC TTG ATA TTG 2208
     Phe His Thr Trp Val Asn Gly Leu Gly Gln His Ala Ser Leu Ile Leu
                         725             730             735
  5
     GCG GCG TTG AAA GAC GGA GCC TTG ACA GTT ACC GAT GTA GCA CAA GCT 2256
     Ala Ala Leu Lys Asp Gly Ala Leu Thr Val Thr Asp Val Ala Gln Ala
                     740             745             750
 10  ATG AAT AAG GAG GAA TCT CTC CTA CAA ATG GCA GCT AAT CAG GTG GAG 2304
     Met Asn Lys Glu Glu Ser Leu Leu Gln Met Ala Ala Asn Gln Val Glu
                     755             760             765

 15  AAG GAT CTA ACA AAA CTG ACC AGT TGG ACA CAG ATT GAC GCT ATT CTG 2352
     Lys Asp Leu Thr Lys Leu Thr Ser Trp Thr Gln Ile Asp Ala Ile Leu
                 770             775             780

     CAA TGG TTA CAG ATG TCT TCG GCC TTG GCG GTT TCT CCA CTG GAT CTG 2400
     Gln Trp Leu Gln Met Ser Ser Ala Leu Ala Val Ser Pro Leu Asp Leu
 20  785             790             795             800

     GCA GGG ATG ATG GCC CTG AAA TAT GGG ATA GAT CAT AAC TAT GCT GCC 2448
     Ala Gly Met Met Ala Leu Lys Tyr Gly Ile Asp His Asn Tyr Ala Ala
                     805             810             815
 25
     TGG CAA GCT GCG GCG GCT GCG CTG ATG GCT GAT CAT GCT AAT CAG GCA 2496
     Trp Gln Ala Ala Ala Ala Ala Leu Met Ala Asp His Ala Asn Gln Ala
                     820             825             830

 30  CAG AAA AAA CTG GAT GAG ACG TTC AGT AAG GCA TTA TGT AAC TAT TAT 2544
     Gln Lys Lys Leu Asp Glu Thr Phe Ser Lys Ala Leu Cys Asn Tyr Tyr
                     835             840             845

     ATT AAT GCT GTT GTC GAT AGT GCT GCT GGA GTA CGT GAT CGT AAC GGT 2592
     Ile Asn Ala Val Val Asp Ser Ala Ala Gly Val Arg Asp Arg Asn Gly
 35                  850             855             860

     TTA TAT ACC TAT TTG CTG ATT GAT AAT CAG GTT TCT GCC GAT GTG ATC 2640
     Leu Tyr Thr Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Asp Val Ile
 40  865             870             875             880

     ACT TCA CGT ATT GCA GAA GCT ATC GCC GGT ATT CAA CTG TAC GTT AAC 2688
     Thr Ser Arg Ile Ala Glu Ala Ile Ala Gly Ile Gln Leu Tyr Val Asn
                     885             890             895
 45
     CGG GCT TTA AAC CGA GAT GAA GGT CAG CTT GCA TCG GAC GTT AGT ACC 2736
     Arg Ala Leu Asn Arg Asp Glu Gly Gln Leu Ala Ser Asp Val Ser Thr
                     900             905             910

 50  CGT CAG TTC TTC ACT GAC TGG GAA CGT TAC AAT AAA CGT TAC AGT ACT 2784
     Arg Gln Phe Phe Thr Asp Trp Glu Arg Tyr Asn Lys Arg Tyr Ser Thr
                     915             920             925

     TGG GCT GGT GTC TCT GAA CTG GTC TAT TAT CCA GAA AAC TAT GTT GAT 2832
     Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr Val Asp
 55  930             935             940

     CCC ACT CAG CGC ATT GGG CAA ACC AAA ATG ATG GAT GCG CTG TTG CAA 2880
     Pro Thr Gln Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln
 60  945             950             955             960

     TCC ATC AAC CAG AGC CAG CTA AAT GCG GAT ACG GTG GAA GAT GCT TTC 2928
     Ser Ile Asn Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe
                     965             970             975
 65
     AAA ACT TAT TTG ACC AGC TTT GAG CAG GTA GCA AAT CTG AAA GTA ATT 2976
     Lys Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile
                     980             985             990

 70  AGT GCT TAC CAC GAT AAT GTG AAT GTG GAT CAA GGA TTA ACT TAT TTT 3024
     Ser Ala Tyr His Asp Asn Val Asn Val Asp Gln Gly Leu Thr Tyr Phe
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SUBSTITUTE SHEET (RULE 26)

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            995              1000              1005

    ATC GGT ATC GAC CAA GCA GCT CCG GGT ACG TAT TAC TGG CGT AGT GTT 3072
    Ile Gly Ile Asp Gln Ala Ala Pro Gly Thr Tyr Tyr Trp Arg Ser Val
5       1010              1015              1020

    GAT CAC AGC AAA TGT GAA AAT GGC AAG TTT GCC GCT AAT GCT TGG GGT 3120
    Asp His Ser Lys Cys Glu Asn Gly Lys Phe Ala Ala Asn Ala Trp Gly
    1025              1030              1035              1040
10
    GAG TGG AAT AAA ATT ACC TGT GCT GTC AAT CCT TGG AAA AAT ATC ATC 3168
    Glu Trp Asn Lys Ile Thr Cys Ala Val Asn Pro Trp Lys Asn Ile Ile
                      1045              1050              1055

15  CGT CCG GTT GTT TAT ATG TCC CGC TTA TAT CTG CTA TGG CTG GAG CAG 3216
    Arg Pro Val Val Tyr Met Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln
                      1060              1065              1070

    CAA TCA AAG AAA AGT GAT GAT GGT AAA ACC ACG ATT TAT CAA TAT AAC 3264
    Gln Ser Lys Lys Ser Asp Asp Gly Lys Thr Thr Ile Tyr Gln Tyr Asn
20      1075              1080              1085

    TTA AAA CTG GCT CAT ATT CGT TAC GAC GGT AGT TGG AAT ACA CCA TTT 3312
    Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Ser Trp Asn Thr Pro Phe
25      1090              1095              1100

    ACT TTT GAT GTG ACA GAA AAG GTA AAA AAT TAC ACG TCG AGT ACT GAT 3360
    Thr Phe Asp Val Thr Glu Lys Val Lys Asn Tyr Thr Ser Ser Thr Asp
    1105              1110              1115              1120
30
    GCT GCT GAA TCT TTA GGG TTG TAT TGT ACT GGT TAT CAA GGG GAA GAC 3408
    Ala Ala Glu Ser Leu Gly Leu Tyr Cys Thr Gly Tyr Gln Gly Glu Asp
                      1125              1130              1135

35  ACT CTA TTA GTT ATG TTC TAT TCG ATG CAG AGT AGT TAT AGC TCC TAT 3456
    Thr Leu Leu Val Met Phe Tyr Ser Met Gln Ser Ser Tyr Ser Ser Tyr
                      1140              1145              1150

    ACC GAT AAT AAT GCG CCG GTC ACT GGG CTA TAT ATT TTC GCT GAT ATG 3504
    Thr Asp Asn Asn Ala Pro Val Thr Gly Leu Tyr Ile Phe Ala Asp Met
40      1155              1160              1165

    TCA TCA GAC AAT ATG ACG AAT GCA CAA GCA ACT AAC TAT TGG AAT AAC 3552
    Ser Ser Asp Asn Met Thr Asn Ala Gln Ala Thr Asn Tyr Trp Asn Asn
45      1170              1175              1180

    AGT TAT CCG CAA TTT GAT ACT GTG ATG GCA GAT CCG GAT AGC GAC AAT 3600
    Ser Tyr Pro Gln Phe Asp Thr Val Met Ala Asp Pro Asp Ser Asp Asn
    1185              1190              1195              1200
50
    AAA AAA GTC ATA ACC AGA AGA GTT AAT AAC CGT TAT GCG GAG GAT TAT 3648
    Lys Lys Val Ile Thr Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr
                      1205              1210              1215

55  GAA ATT CCT TCC TCT GTG ACA AGT AAC AGT AAT TAT TCT TGG GGT GAT 3696
    Glu Ile Pro Ser Ser Val Thr Ser Asn Ser Asn Tyr Ser Trp Gly Asp
                      1220              1225              1230

    CAC AGT TTA ACC ATG CTT TAT GGT GGT AGT GTT CCT AAT ATT ACT TTT 3744
    His Ser Leu Thr Met Leu Tyr Gly Gly Ser Val Pro Asn Ile Thr Phe
60      1235              1240              1245

    GAA TCG GCG GCA GAA GAT TTA AGG CTA TCT ACC AAT ATG GCA TTG AGT 3792
    Glu Ser Ala Ala Glu Asp Leu Arg Leu Ser Thr Asn Met Ala Leu Ser
65      1250              1255              1260

    ATT ATT CAT AAT GGA TAT GCG GGA ACC CGC CGT ATA CAA TGT AAT CTT 3840
    Ile Ile His Asn Gly Tyr Ala Gly Thr Arg Arg Ile Gln Cys Asn Leu
    1265              1270              1275              1280
70
    ATG AAA CAA TAC GCT TCA TTA GGT GAT AAA TTT ATA ATT TAT GAT TCA 3888
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SUBSTITUTE SHEET (RULE 26)

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            Met Lys Gln Tyr Ala Ser Leu Gly Asp Lys Phe Ile Ile Tyr Asp Ser
                         1285                1290                1295

    5       TCA TTT GAT GAT GCA AAC CGT TTT AAT CTG GTG CCA TTG TTT AAA TTC 3936
            Ser Phe Asp Asp Ala Asn Arg Phe Asn Leu Val Pro Leu Phe Lys Phe
                         1300                1305                1310

            GGA AAA GAC GAG AAC TCA GAT GAT AGT ATT TGT ATA TAT AAT GAA AAC 3984
            Gly Lys Asp Glu Asn Ser Asp Asp Ser Ile Cys Ile Tyr Asn Glu Asn
    10                1315                1320                1325

            CCT TCC TCT GAA GAT AAG AAG TGG TAT TTT TCT TCG AAA GAT GAC AAT 4032
            Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe Ser Ser Lys Asp Asp Asn
    15                1330                1335                1340

            AAA ACA GCG GAT TAT AAT GGT GGA ACT CAA TGT ATA GAT GCT GGA ACC 4080
            Lys Thr Ala Asp Tyr Asn Gly Gly Thr Gln Cys Ile Asp Ala Gly Thr
            1345                1350                1355                1360

    20      AGT AAC AAA GAT TTT TAT TAT AAT CTC CAG GAG ATT GAA GTA ATT AGT 4128
            Ser Asn Lys Asp Phe Tyr Tyr Asn Leu Gln Glu Ile Glu Val Ile Ser
                         1365                1370                1375

            GTT ACT GGT GGG TAT TGG TCG AGT TAT AAA ATA TCC AAC CCG ATT AAT 4176
    25      Val Thr Gly Gly Tyr Trp Ser Ser Tyr Lys Ile Ser Asn Pro Ile Asn
                         1380                1385                1390

            ATC AAT ACG GGC ATT GAT AGT GCT AAA GTA AAA GTC ACC GTA AAA GCG 4224
            Ile Asn Thr Gly Ile Asp Ser Ala Lys Val Lys Val Thr Val Lys Ala
    30                1395                1400                1405

            GGT GGT GAC GAT CAA ATC TTT ACT GCT GAT AAT AGT ACC TAT GTT CCT 4272
            Gly Gly Asp Asp Gln Ile Phe Thr Ala Asp Asn Ser Thr Tyr Val Pro
            1410                1415                1420
    35
            CAG CAA CCG GCA CCC AGT TTT GAG GAG ATG ATT TAT CAG TTC AAT AAC 4320
            Gln Gln Pro Ala Pro Ser Phe Glu Glu Met Ile Tyr Gln Phe Asn Asn
            1425                1430                1435                1440

    40      CTG ACA ATA GAT TGT AAG AAT TTA AAT TTC ATC GAC AAT CAG GCA CAT 4368
            Leu Thr Ile Asp Cys Lys Asn Leu Asn Phe Ile Asp Asn Gln Ala His
                         1445                1450                1455

            ATT GAG ATT GAT TTC ACC GCT ACG GCA CAA GAT GGC CGA TTC TTG GGT 4416
    45      Ile Glu Ile Asp Phe Thr Ala Thr Ala Gln Asp Gly Arg Phe Leu Gly
                         1460                1465                1470

            GCA GAA ACT TTT ATT ATC CCG GTA ACT AAA AAA GTT CTC GGT ACT GAG 4464
            Ala Glu Thr Phe Ile Ile Pro Val Thr Lys Lys Val Leu Gly Thr Glu
    50                1475                1480                1485

            AAC GTG ATT GCG TTA TAT AGC GAA AAT AAC GGT GTT CAA TAT ATG CAA 4512
            Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly Val Gln Tyr Met Gln
    55                1490                1495                1500

            ATT GGC GCA TAT CGT ACC CGT TTG AAT ACG TTA TTC GCT CAA CAG TTG 4560
            Ile Gly Ala Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Gln Gln Leu
            1505                1510                1515                1520

    60      GTT AGC CGT GCT AAT CGT GGC ATT GAT GCA GTG CTC AGT ATG GAA ACT 4608
            Val Ser Arg Ala Asn Arg Gly Ile Asp Ala Val Leu Ser Met Glu Thr
                         1525                1530                1535

            CAG AAT ATT CAG GAA CCG CAA TTA GGA GCG GGC ACA TAT GTG CAG CTT 4656
    65      Gln Asn Ile Gln Glu Pro Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
                         1540                1545                1550

            GTG TTG GAT AAA TAT GAT GAG TCT ATT CAT GGC ACT AAT AAA AGC TTT 4704
            Val Leu Asp Lys Tyr Asp Glu Ser Ile His Gly Thr Asn Lys Ser Phe
    70                1555                1560                1565
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SUBSTITUTE SHEET (RULE 26)

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        GCT ATT GAA TAT GTT GAT ATA TTT AAA GAG AAC GAT AGT TTT GTG ATT 4752
        Ala Ile Glu Tyr Val Asp Ile Phe Lys Glu Asn Asp Ser Phe Val Ile
            1570                1575                1580

  5     TAT CAA GGA GAA CTT AGC GAA ACA AGT CAA ACT GTT GTG AAA GTT TTC 4800
        Tyr Gln Gly Glu Leu Ser Glu Thr Ser Gln Thr Val Val Lys Val Phe
        1585                1590                1595                1600

        TTA TCC TAT TTT ATA GAG GCG ACT GGA AAT AAG AAC CAC TTA TGG GTA 4848
 10     Leu Ser Tyr Phe Ile Glu Ala Thr Gly Asn Lys Asn His Leu Trp Val
                        1605                1610                1615

        CGT GCT AAA TAC CAA AAG GAA ACG ACT GAT AAG ATC TTG TTC GAC CGT 4896
        Arg Ala Lys Tyr Gln Lys Glu Thr Thr Asp Lys Ile Leu Phe Asp Arg
 15                     1620                1625                1630

        ACT GAT GAG AAA GAT CCG CAC GGT TGG TTT CTC AGC GAC GAT CAC AAG 4944
        Thr Asp Glu Lys Asp Pro His Gly Trp Phe Leu Ser Asp Asp His Lys
                    1635                1640                1645
 20
        ACC TTT AGT GGT CTC TCT TCC GCA CAG GCA TTA AAG AAC GAC AGT GAA 4992
        Thr Phe Ser Gly Leu Ser Ser Ala Gln Ala Leu Lys Asn Asp Ser Glu
            1650                1655                1660

 25     CCG ATG GAT TTC TCT GGC GCC AAT GCT CTC TAT TTC TGG GAA CTG TTC 5040
        Pro Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe
        1665                1670                1675                1680

        TAT TAC ACG CCG ATG ATG ATG GCT CAT CGT TTG TTG CAG GAA CAG AAT 5088
 30     Tyr Tyr Thr Pro Met Met Met Ala His Arg Leu Leu Gln Glu Gln Asn
                        1685                1690                1695

        TTT GAT GCG GCG AAC CAT TGG TTC CGT TAT GTC TGG AGT CCA TCC GGT 5136
        Phe Asp Ala Ala Asn His Trp Phe Arg Tyr Val Trp Ser Pro Ser Gly
 35                     1700                1705                1710

        TAT ATC GTT GAT GGT AAA ATT GCT ATC TAC CAC TGG AAC GTG CGA CCG 5184
        Tyr Ile Val Asp Gly Lys Ile Ala Ile Tyr His Trp Asn Val Arg Pro
                1715                1720                1725
 40
        CTG GAA GAA GAC ACC AGT TGG AAT GCA CAA CAA CTG GAC TCC ACC GAT 5232
        Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp
            1730                1735                1740

 45     CCA GAT GCT GTA GCC CAA GAT GAT CCG ATG CAC TAC AAG GTG GCT ACC 5280
        Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr
        1745                1750                1755                1760

        TTT ATG GCG ACG TTG GAT CTG CTA ATG GCC CGT GGT GAT GCT GCT TAC 5328
 50     Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr
                        1765                1770                1775

        CGC CAG TTA GAG CGT GAT ACG TTG GCT GAA GCT AAA ATG TGG TAT ACA 5376
        Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr
 55                     1780                1785                1790

        CAG GCG CTT AAT CTG TTG GGT GAT GAG CCA CAA GTG ATG CTG AGT ACG 5424
        Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr
                    1795                1800                1805
 60
        ACT TGG GCT AAT CCA ACA TTG GGT AAT GCT GCT TCA AAA ACC ACA CAG 5472
        Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln
            1810                1815                1820

 65     CAG GTT CGT CAG CAA GTG CTT ACC CAG TTG CGT CTC AAT AGC AGG GTA 5520
        Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val
        1825                1830                1835                1840

        AAA ACC CCG TTG     5532
 70     Lys Thr Pro Leu
                    1844
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SUBSTITUTE SHEET (RULE 26)

(2) INFORMATION FOR SEQ ID NO:53:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1844 amino acids
        (B) TYPE: amino acids
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53 (TcbA$_{ii}$):

Features	From	To	Description
Peptide	1	1844	TcbA$_{ii}$ peptide
Fragment	1	11	(SEQ ID NO:1)
Fragment	978	990	(SEQ ID NO:23)
Fragment	1387	1401	(SEQ ID NO:22)
Fragment	1484	1505	(SEQ ID NO:24)
Fragment	1527	1552	(SEQ ID NO:21)

```
Phe Ile Gln Gly Tyr Ser Asp Leu Phe Gly Asn Arg Ala Asp Asn Tyr
 1           5               10              15

Ala Ala Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala Tyr Leu
        20              25              30

Thr Glu Leu Tyr Arg Glu Ala Lys Asn Leu His Asp Ser Ser Ser Ile
        35              40              45

Tyr Tyr Leu Asp Lys Arg Arg Pro Asp Leu Ala Ser Leu Met Leu Ser
    50              55              60

Gln Lys Asn Met Asp Glu Glu Ile Ser Thr Leu Ala Leu Ser Asn Glu
65              70              75              80

Leu Cys Leu Ala Gly Ile Glu Thr Lys Thr Gly Lys Ser Gln Asp Glu
                85              90              95

Val Met Asp Met Leu Ser Thr Tyr Arg Leu Ser Gly Glu Thr Pro Tyr
            100             105             110

His His Ala Tyr Glu Thr Val Arg Glu Ile Val His Glu Arg Asp Pro
        115             120             125

Gly Phe Arg His Leu Ser Gln Ala Pro Ile Val Ala Ala Lys Leu Asp
        130             135             140

Pro Val Thr Leu Leu Gly Ile Ser Ser His Ile Ser Pro Glu Leu Tyr
145             150             155             160

Asn Leu Leu Ile Glu Glu Ile Pro Glu Lys Asp Glu Ala Ala Leu Asp
            165             170             175

Thr Leu Tyr Lys Thr Asn Phe Gly Asp Ile Thr Thr Ala Gln Leu Met
            180             185             190

Ser Pro Ser Tyr Leu Ala Arg Tyr Tyr Gly Val Ser Pro Glu Asp Ile
        195             200             205

Ala Tyr Val Thr Thr Ser Leu Ser His Val Gly Tyr Ser Ser Asp Ile
        210             215             220

Leu Val Ile Pro Leu Val Asp Gly Val Gly Lys Met Glu Val Val Arg
225             230             235             240

Val Thr Arg Thr Pro Ser Asp Asn Tyr Thr Ser Gln Thr Asn Tyr Ile
            245             250             255
```

SUBSTITUTE SHEET (RULE 26)

```
         Glu Leu Tyr Pro Gln Gly Gly Asp Asn Tyr.Leu Ile Lys Tyr Asn Leu
                     260             265                     270
   5     Ser Asn Ser Phe Gly Leu Asp Asp Phe Tyr Leu Gln Tyr Lys Asp Gly
                 275             280                 285

         Ser Ala Asp Trp Thr Glu Ile Ala His Asn Pro Tyr Pro Asp Met Val
                 290             295                 300

  10     Ile Asn Gln Lys Tyr Glu Ser Gln Ala Thr Ile Lys Arg Ser Asp Ser
         305             310                 315                 320

         Asp Asn Ile Leu Ser Ile Gly Leu Gln Arg Trp His Ser Gly Ser Tyr
  15                     325             330                 335

         Asn Phe Ala Ala Ala Asn Phe Lys Ile Asp Gln Tyr Ser Pro Lys Ala
                     340             345                 350

  20     Phe Leu Leu Lys Met Asn Lys Ala Ile Arg Leu Leu Lys Ala Thr Gly
                 355             360                 365

         Leu Ser Phe Ala Thr Leu Glu Arg Ile Val Asp Ser Val Asn Ser Thr
                 370             375                 380

  25     Lys Ser Ile Thr Val Glu Val Leu Asn Lys Val Tyr Arg Val Lys Phe
         385              390                 395                 400

         Tyr Ile Asp Arg Tyr Gly Ile Ser Glu Glu Thr Ala Ala Ile Leu Ala
  30                     405             410                     415

         Asn Ile Asn Ile Ser Gln Gln Ala Val Gly Asn Gln Leu Ser Gln Phe
                     420             425                 430

  35     Glu Gln Leu Phe Asn His Pro Pro Leu Asn Gly Ile Arg Tyr Glu Ile
                 435             440                 445

         Ser Glu Asp Asn Ser Lys His Leu Pro Asn Pro Asp Leu Asn Leu Lys
                 450             455                 460

  40     Pro Asp Ser Thr Gly Asp Asp Gln Arg Lys Ala Val Leu Lys Arg Ala
         465             470                 475                 480

         Phe Gln Val Asn Ala Ser Glu Leu Tyr Gln Met Leu Leu Ile Thr Asp
                     485             490                 495
  45
         Arg Lys Glu Asp Gly Val Ile Lys Asn Asn Leu Glu Asn Leu Ser Asp
                     500             505                 510

         Leu Tyr Leu Val Ser Leu Leu Ala Gln Ile His Asn Leu Thr Ile Ala
  50                 515             520                 525

         Glu Leu Asn Ile Leu Leu Val Ile Cys Gly Tyr Gly Asp Thr Asn Ile
                 530             535                 540

  55     Tyr Gln Ile Thr Asp Asp Asn Leu Ala Lys Ile Val Glu Thr Leu Leu
         545             550                 555                 560

         Trp Ile Thr Gln Trp Leu Lys Thr Gln Lys Trp Thr Val Thr Asp Leu
                     565             570                 575
  60
         Phe Leu Met Thr Thr Ala Thr Tyr Ser Thr Thr Leu Thr Pro Glu Ile
                     580             585                 590

         Ser Asn Leu Thr Ala Thr Leu Ser Ser Thr Leu His Gly Lys Glu Ser
  65                 595             600                 605

         Leu Ile Gly Glu Asp Leu Lys Arg Ala Met Ala Pro Cys Phe Thr Ser
                 610             615                 620

  70     Ala Leu His Leu Thr Ser Gln Glu Val Ala Tyr Asp Leu Leu Leu Trp
         625             630                 635                 640
```

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                        SUBSTITUTE SHEET (RULE 26)

```
       Ile Asp Gln Ile Gln Pro Ala Gln Ile Thr Val Asp Gly Phe Trp Glu
                       645             650                 655

  5    Glu Val Gln Thr Thr Pro Thr Ser Leu Lys Val Ile Thr Phe Ala Gln
                       660             665                 670

       Val Leu Ala Gln Leu Ser Leu Ile Tyr Arg Arg Ile Gly Leu Ser Glu
                       675             680                 685

 10    Thr Glu Leu Ser Leu Ile Val Thr Gln Ser Ser Leu Leu Val Ala Gly
                       690             695                 700

       Lys Ser Ile Leu Asp His Gly Leu Leu Thr Leu Met Ala Leu Glu Gly
 15    705             710                 715                 720

       Phe His Thr Trp Val Asn Gly Leu Gly Gln His Ala Ser Leu Ile Leu
                       725             730                 735

 20    Ala Ala Leu Lys Asp Gly Ala Leu Thr Val Thr Asp Val Ala Gln Ala
                       740             745                 750

       Met Asn Lys Glu Glu Ser Leu Leu Gln Met Ala Ala Asn Gln Val Glu
                       755             760                 765
 25
       Lys Asp Leu Thr Lys Leu Thr Ser Trp Thr Gln Ile Asp Ala Ile Leu
                       770             775                 780

       Gln Trp Leu Gln Met Ser Ser Ala Leu Ala Val Ser Pro Leu Asp Leu
 30    785             790                 795                 800

       Ala Gly Met Met Ala Leu Lys Tyr Gly Ile Asp His Asn Tyr Ala Ala
                       805             810                 815

 35    Trp Gln Ala Ala Ala Ala Leu Met Ala Asp His Ala Asn Gln Ala
                       820             825                 830

       Gln Lys Lys Leu Asp Glu Thr Phe Ser Lys Ala Leu Cys Asn Tyr Tyr
                       835             840                 845
 40
       Ile Asn Ala Val Val Asp Ser Ala Ala Gly Val Arg Asp Arg Asn Gly
                       850             855                 860

       Leu Tyr Thr Tyr Leu Leu Ile Asp Asn Gln Val Ser Ala Asp Val Ile
 45    865             870                 875                 880

       Thr Ser Arg Ile Ala Glu Ala Ile Ala Gly Ile Gln Leu Tyr Val Asn
                       885             890                 895

 50    Arg Ala Leu Asn Arg Asp Glu Gly Gln Leu Ala Ser Asp Val Ser Thr
                       900             905                 910

       Arg Gln Phe Phe Thr Asp Trp Glu Arg Tyr Asn Lys Arg Tyr Ser Thr
                       915             920                 925
 55
       Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr Val Asp
                       930             935                 940

       Pro Thr Gln Arg Ile Gly Gln Thr Lys Met Met Asp Ala Leu Leu Gln
 60    945             950                 955                 960

       Ser Ile Asn Gln Ser Gln Leu Asn Ala Asp Thr Val Glu Asp Ala Phe
                       965             970                 975

 65    Lys Thr Tyr Leu Thr Ser Phe Glu Gln Val Ala Asn Leu Lys Val Ile
                       980             985                 990

       Ser Ala Tyr His Asp Asn Val Asn Val Asp Gln Gly Leu Thr Tyr Phe
                       995             1000                1005
 70
       Ile Gly Ile Asp Gln Ala Ala Pro Gly Thr Tyr Tyr Trp Arg Ser Val
```

SUBSTITUTE SHEET (RULE 26)

```
              1010                    1015                  1020

        Asp His Ser Lys Cys Glu Asn Gly Lys Phe Ala Ala Asn Ala Trp Gly
        1025                    1030                1035              1040
   5
        Glu Trp Asn Lys Ile Thr Cys Ala Val Asn Pro Trp Lys Asn Ile Ile
                        1045            1050              1055
  10
        Arg Pro Val Val Tyr Met Ser Arg Leu Tyr Leu Leu Trp Leu Glu Gln
                    1060            1065            1070
  10
        Gln Ser Lys Lys Ser Asp Asp Gly Lys Thr Thr Ile Tyr Gln Tyr Asn
                1075                1080              1085
  15
        Leu Lys Leu Ala His Ile Arg Tyr Asp Gly Ser Trp Asn Thr Pro Phe
                1090            1095            1100
        Thr Phe Asp Val Thr Glu Lys Val Lys Asn Tyr Thr Ser Ser Thr Asp
        1105                1110              1115                 1120
  20
        Ala Ala Glu Ser Leu Gly Leu Tyr Cys Thr Gly Tyr Gln Gly Glu Asp
                  1125              1130              1135
  25
        Thr Leu Leu Val Met Phe Tyr Ser Met Gln Ser Ser Tyr Ser Ser Tyr
                    1140            1145            1150
  25
        Thr Asp Asn Asn Ala Pro Val Thr Gly Leu Tyr Ile Phe Ala Asp Met
                1155            1160            1165
  30
        Ser Ser Asp Asn Met Thr Asn Ala Gln Ala Thr Asn Tyr Trp Asn Asn
                1170            1175            1180
        Ser Tyr Pro Gln Phe Asp Thr Val Met Ala Asp Pro Asp Ser Asp Asn
        1185            1190            1195                 1200
  35
        Lys Lys Val Ile Thr Arg Arg Val Asn Asn Arg Tyr Ala Glu Asp Tyr
                    1205            1210            1215
        Glu Ile Pro Ser Ser Val Thr Ser Asn Ser Asn Tyr Ser Trp Gly Asp
  40            1220            1225            1230
        His Ser Leu Thr Met Leu Tyr Gly Gly Ser Val Pro Asn Ile Thr Phe
                1235            1240            1245
  45
        Glu Ser Ala Ala Glu Asp Leu Arg Leu Ser Thr Asn Met Ala Leu Ser
                1250            1255            1260
        Ile Ile His Asn Gly Tyr Ala Gly Thr Arg Arg Ile Gln Cys Asn Leu
        1265            1270            1275              1280
  50
        Met Lys Gln Tyr Ala Ser Leu Gly Asp Lys Phe Ile Ile Tyr Asp Ser
                    1285            1290            1295
        Ser Phe Asp Asp Ala Asn Arg Phe Asn Leu Val Pro Leu Phe Lys Phe
  55            1300            1305            1310
        Gly Lys Asp Glu Asn Ser Asp Asp Ser Ile Cys Ile Tyr Asn Glu Asn
                1315            1320            1325
        Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe Ser Ser Lys Asp Asp Asn
  60            1330            1335            1340
        Lys Thr Ala Asp Tyr Asn Gly Gly Thr Gln Cys Ile Asp Ala Gly Thr
        1345            1350            1355              1360
  65
        Ser Asn Lys Asp Phe Tyr Tyr Asn Leu Gln Glu Ile Glu Val Ile Ser
                    1365            1370            1375
        Val Thr Gly Gly Tyr Trp Ser Ser Tyr Lys Ile Ser Asn Pro Ile Asn
  70            1380            1385            1390
```

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Ile Asn Thr Gly Ile Asp Ser Ala Lys Val. Lys Val Thr Val Lys Ala
    1395   1400   1405

5   Gly Gly Asp Asp Gln Ile Phe Thr Ala Asp Asn Ser Thr Tyr Val Pro
        1410   1415   1420

Gln Gln Pro Ala Pro Ser Phe Glu Glu Met Ile Tyr Gln Phe Asn Asn
1425   1430   1435   1440

10 Leu Thr Ile Asp Cys Lys Asn Leu Asn Phe Ile Asp Asn Gln Ala His
            1445   1450   1455

Ile Glu Ile Asp Phe Thr Ala Thr Ala Gln Asp Gly Arg Phe Leu Gly
        1460   1465   1470
15

Ala Glu Thr Phe Ile Ile Pro Val Thr Lys Lys Val Leu Gly Thr Glu
        1475   1480   1485

Asn Val Ile Ala Leu Tyr Ser Glu Asn Asn Gly Val Gln Tyr Met Gln
20   1490   1495   1500

Ile Gly Ala Tyr Arg Thr Arg Leu Asn Thr Leu Phe Ala Gln Gln Leu
1505   1510   1515   1520

25 Val Ser Arg Ala Asn Arg Gly Ile Asp Ala Val Leu Ser Met Glu Thr
            1525   1530   1535

Gln Asn Ile Gln Glu Pro Gln Leu Gly Ala Gly Thr Tyr Val Gln Leu
        1540   1545   1550
30

Val Leu Asp Lys Tyr Asp Glu Ser Ile His Gly Thr Asn Lys Ser Phe
        1555   1560   1565

Ala Ile Glu Tyr Val Asp Ile Phe Lys Glu Asn Asp Ser Phe Val Ile
35   1570   1575   1580

Tyr Gln Gly Glu Leu Ser Glu Thr Ser Gln Thr Val Val Lys Val Phe
1585   1590   1595   1600

40 Leu Ser Tyr Phe Ile Glu Ala Thr Gly Asn Lys Asn His Leu Trp Val
            1605   1610   1615

Arg Ala Lys Tyr Gln Lys Glu Thr Thr Asp Lys Ile Leu Phe Asp Arg
        1620   1625   1630
45

Thr Asp Glu Lys Asp Pro His Gly Trp Phe Leu Ser Asp Asp His Lys
        1635   1640   1645

Thr Phe Ser Gly Leu Ser Ser Ala Gln Ala Leu Lys Asn Asp Ser Glu
50   1650   1655   1660

Pro Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe
1665   1670   1675   1680

55 Tyr Tyr Thr Pro Met Met Met Ala His Arg Leu Leu Gln Glu Gln Asn
            1685   1690   1695

Phe Asp Ala Ala Asn His Trp Phe Arg Tyr Val Trp Ser Pro Ser Gly
        1700   1705   1710
60

Tyr Ile Val Asp Gly Lys Ile Ala Ile Tyr His Trp Asn Val Arg Pro
        1715   1720   1725

Leu Glu Glu Asp Thr Ser Trp Asn Ala Gln Gln Leu Asp Ser Thr Asp
65   1730   1735   1740

Pro Asp Ala Val Ala Gln Asp Asp Pro Met His Tyr Lys Val Ala Thr
1745   1750   1755   1760

70 Phe Met Ala Thr Leu Asp Leu Leu Met Ala Arg Gly Asp Ala Ala Tyr
            1765   1770   1775

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SUBSTITUTE SHEET (RULE 26)

```
       Arg Gln Leu Glu Arg Asp Thr Leu Ala Glu Ala Lys Met Trp Tyr Thr
                 1780                    1785                1790
  5    Gln Ala Leu Asn Leu Leu Gly Asp Glu Pro Gln Val Met Leu Ser Thr
                 1795                    1800            1805
       Thr Trp Ala Asn Pro Thr Leu Gly Asn Ala Ala Ser Lys Thr Thr Gln
 10              1810                    1815            1820
       Gln Val Arg Gln Gln Val Leu Thr Gln Leu Arg Leu Asn Ser Arg Val
                 1825                    1830            1835            1840
 15    Lys Thr Pro Leu
                 1844
```

(2) INFORMATION FOR SEQ ID NO:54:

 20   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 1722 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
 25   (ii) MOLECULE TYPE: DNA (genomic)

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54 (*tcbAiii* coding
region):

```
 30    CTA GGA ACA GCC AAT TCC CTG ACC GCT TTA TTC CTG CCG CAG GAA AAT  48
       Leu Gly Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn
        1               5                   10                  15
       AGC AAG CTC AAA GGC TAC TGG CGG ACA CTG GCG CAG CGT ATG TTT AAT  96
 35    Ser Lys Leu Lys Gly Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn
                     20                  25                  30
       TTA CGT CAT AAT CTG TCG ATT GAC GGC CAG CCG CTC TCC TTG CCG CTG 144
       Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu
 40                35                  40                  45
       TAT GCT AAA CCG GCT GAT CCA AAA GCT TTA CTG AGT GCG GCG GTT TCA 192
       Tyr Ala Lys Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser
            50                  55                  60
 45    GCT TCT CAA GGG GGA GCC GAC TTG CCG AAG GCG CCG CTG ACT ATT CAC 240
       Ala Ser Gln Gly Gly Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His
       65                  70                  75                  80
 50    CGC TTC CCT CAA ATG CTA GAA GGG GCA CGG GGC TTG GTT AAC CAG CTT 288
       Arg Phe Pro Gln Met Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu
                         85                  90                  95
       ATA CAG TTC GGT AGT TCA CTA TTG GGG TAC AGT GAG CGT CAG GAT GCG 336
 55    Ile Gln Phe Gly Ser Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala
                         100                 105                 110
       GAA GCT ATG AGT CAA CTA CTG CAA ACC CAA GCC AGC GAG TTA ATA CTG 384
       Glu Ala Met Ser Gln Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu
 60                      115                 120                 125
       ACC AGT ATT CGT ATG CAG GAT AAC CAA TTG GCA GAG CTG GAT TCG GAA 432
       Thr Ser Ile Arg Met Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu
                 130                 135                 140
 65    AAA ACC GCC TTG CAA GTC TCT TTA GCT GGA GTG CAA CAA CGG TTT GAC 480
       Lys Thr Ala Leu Gln Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp
       145                 150                 155                 160
```

SUBSTITUTE SHEET (RULE 26)

```
    AGC TAT AGC CAA CTG TAT GAG GAG AAC ATC AAC GCA GGT GAG CAG CGA 528
    Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg
                     165             170             175

 5  GCG CTG GCG TTA CGC TCA GAA TCT GCT ATT GAG TCT CAG GGA GCG CAG 576
    Ala Leu Ala Leu Arg Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln
                     180             185             190

10  ATT TCC CGT ATG GCA GGC GCG GGT GTT GAT ATG GCA CCA AAT ATC TTC 624
    Ile Ser Arg Met Ala Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe
                     195             200             205

    GGC CTG GCT GAT GGC GGC ATG CAT TAT GGT GCT ATT GCC TAT GCC ATC 672
    Gly Leu Ala Asp Gly Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile
15                   210             215             220

    GCT GAC GGT ATT GAG TTG AGT GCT TCT GCC AAG ATG GTT GAT GCG GAG 720
    Ala Asp Gly Ile Glu Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu
    225                  230             235                 240
20
    AAA GTT GCT CAG TCG GAA ATA TAT CGC CGT CGC CGT CAA GAA TGG AAA 768
    Lys Val Ala Gln Ser Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys
                     245             250             255

25  ATT CAG CGT GAC AAC GCA CAA GCG GAG ATT AAC CAG TTA AAC GCG CAA 816
    Ile Gln Arg Asp Asn Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln
                     260             265             270

    CTG GAA TCA CTG TCT ATT CGC CGT GAA GCC GCT GAA ATG CAA AAA GAG 864
30  Leu Glu Ser Leu Ser Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu
                     275             280             285

    TAC CTG AAA ACC CAG CAA GCT CAG GCG CAG GCA CAA CTT ACT TTC TTA 912
    Tyr Leu Lys Thr Gln Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu
35                   290             295             300

    AGA AGC AAA TTC AGT AAT CAA GCG TTA TAT AGT TGG TTA CGA GGG CGT 960
    Arg Ser Lys Phe Ser Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg
    305                  310             315                 320
40
    TTG TCA GGT ATT TAT TTC CAG TTC TAT GAC TTG GCC GTA TCA CGT TGC 1008
    Leu Ser Gly Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys
                     325             330             335

45  CTG ATG GCA GAG CAA TCC TAT CAA TGG GAA GCT AAT GAT AAT TCC ATT 1056
    Leu Met Ala Glu Gln Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile
                     340             345             350

    AGC TTT GTC AAA CCG GGT GCA TGG CAA GGA ACT TAC GCC GGC TTA TTG 1104
50  Ser Phe Val Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
                     355             360             365

    TGT GGA GAA GCT TTG ATA CAA AAT CTG GCA CAA ATG GAA GAG GCA TAT 1152
    Cys Gly Glu Ala Leu Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr
55  370                  375             380

    CTG AAA TGG GAA TCT CGC GCT TTG GAA GTA GAA CGC ACG GTT TCA TTG 1200
    Leu Lys Trp Glu Ser Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
    385                  390             395                 400
60
    GCA GTG GTT TAT GAT TCA CTG GAA GGT AAT GAT CGT TTT AAT TTA GCG 1248
    Ala Val Val Tyr Asp Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala
                     405             410             415

65  GAA CAA ATA CCT GCA TTA TTG GAT AAG GGG GAG GGA ACA GCA GGA ACT 1296
    Glu Gln Ile Pro Ala Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr
                     420             425             430

    AAA GAA AAT GGG TTA TCA TTG GCT AAT GCT ATC CTG TCA GCT TCG GTC 1344
70  Lys Glu Asn Gly Leu Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val
                     435             440             445
```

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```
AAA TTG TCC GAC TTG AAA CTG GGA ACG GAT TAT CCA GAC AGT ATC GTT 1392
Lys Leu Ser Asp Leu Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val
    450             455             460

GGT AGC AAC AAG GTT CGT CGT ATT AAG CAA ATC AGT GTT TCG CTA CCT 1440
Gly Ser Asn Lys Val Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro
465             470             475             480

GCA TTG GTT GGG CCT TAT CAG GAT GTT CAG GCT ATG CTC AGC TAT GGT 1488
Ala Leu Val Gly Pro Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly
                485             490             495

GGC AGT ACT CAA TTG CCG AAA GGT TGT TCA GCG TTG GCT GTG TCT CAT 1536
Gly Ser Thr Gln Leu Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His
            500             505             510

GGT ACC AAT GAT AGT GGT CAG TTC CAG TTG GAT TTC AAT GAC GGC AAA 1584
Gly Thr Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys
            515             520             525

TAC CTG CCA TTT GAA GGT ATT GCT CTT GAT GAT CAG GGT ACA CTG AAT 1632
Tyr Leu Pro Phe Glu Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn
    530             535             540

CTT CAA TTT CCG AAT GCT ACC GAC AAG CAG AAA GCA ATA TTG CAA ACT 1680
Leu Gln Phe Pro Asn Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr
545             550             555             560

ATG AGC GAT ATT ATT TTG CAT ATT CGT TAT ACC ATC CGT TAA      1722
Met Ser Asp Ile Ile Leu His Ile Arg Tyr Thr Ile Arg •••
            565             570         573
```

(2) INFORMATION FOR SEQ ID NO:55:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 573 amino acids
        (B) TYPE: amino acids
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55 (TcbA$_{iii}$):

```
Leu Gly Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Glu Asn
 1               5               10              15

Ser Lys Leu Lys Gly Tyr Trp Arg Thr Leu Ala Gln Arg Met Phe Asn
            20              25              30

Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Pro Leu
        35              40              45

Tyr Ala Lys Pro Ala Asp Pro Lys Ala Leu Leu Ser Ala Ala Val Ser
    50              55              60

Ala Ser Gln Gly Gly Ala Asp Leu Pro Lys Ala Pro Leu Thr Ile His
65              70              75              80

Arg Phe Pro Gln Met Leu Glu Gly Ala Arg Gly Leu Val Asn Gln Leu
            85              90              95

Ile Gln Phe Gly Ser Ser Leu Leu Gly Tyr Ser Glu Arg Gln Asp Ala
            100             105             110

Glu Ala Met Ser Gln Leu Leu Gln Thr Gln Ala Ser Glu Leu Ile Leu
        115             120             125
```

```
        Thr Ser Ile Arg Met Gln Asp Asn Gln Leu Ala Glu Leu Asp Ser Glu
            130             135             140
   5    Lys Thr Ala Leu Gln Val Ser Leu Ala Gly Val Gln Gln Arg Phe Asp
        145             150             155             160
        Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala Gly Glu Gln Arg
                        165             170             175
  10    Ala Leu Ala Leu Arg Ser Glu Ser Ala Ile Glu Ser Gln Gly Ala Gln
                    180             185             190
        Ile Ser Arg Met Ala Gly Ala Gly Val Asp Met Ala Pro Asn Ile Phe
                195             200             205
  15
        Gly Leu Ala Asp Gly Gly Met His Tyr Gly Ala Ile Ala Tyr Ala Ile
            210             215             220
        Ala Asp Gly Ile Glu Leu Ser Ala Ser Ala Lys Met Val Asp Ala Glu
  20    225             230             235             240
        Lys Val Ala Gln Ser Glu Ile Tyr Arg Arg Arg Arg Gln Glu Trp Lys
                        245             250             255
  25    Ile Gln Arg Asp Asn Ala Gln Ala Glu Ile Asn Gln Leu Asn Ala Gln
                    260             265             270
        Leu Glu Ser Leu Ser Ile Arg Arg Glu Ala Ala Glu Met Gln Lys Glu
                275             280             285
  30
        Tyr Leu Lys Thr Gln Gln Ala Gln Ala Gln Ala Gln Leu Thr Phe Leu
            290             295             300
        Arg Ser Lys Phe Ser Asn Gln Ala Leu Tyr Ser Trp Leu Arg Gly Arg
  35    305             310             315             320
        Leu Ser Gly Ile Tyr Phe Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys
                        325             330             335
  40    Leu Met Ala Glu Gln Ser Tyr Gln Trp Glu Ala Asn Asp Asn Ser Ile
                    340             345             350
        Ser Phe Val Lys Pro Gly Ala Trp Gln Gly Thr Tyr Ala Gly Leu Leu
                355             360             365
  45
        Cys Gly Glu Ala Leu Ile Gln Asn Leu Ala Gln Met Glu Glu Ala Tyr
            370             375             380
        Leu Lys Trp Glu Ser Arg Ala Leu Glu Val Glu Arg Thr Val Ser Leu
  50    385             390             395             400
        Ala Val Val Tyr Asp Ser Leu Glu Gly Asn Asp Arg Phe Asn Leu Ala
                        405             410             415
  55    Glu Gln Ile Pro Ala Leu Leu Asp Lys Gly Glu Gly Thr Ala Gly Thr
                    420             425             430
        Lys Glu Asn Gly Leu Ser Leu Ala Asn Ala Ile Leu Ser Ala Ser Val
                435             440             445
  60
        Lys Leu Ser Asp Leu Lys Leu Gly Thr Asp Tyr Pro Asp Ser Ile Val
            450             455             460
        Gly Ser Asn Lys Val Arg Arg Ile Lys Gln Ile Ser Val Ser Leu Pro
  65    465             470             475             480
        Ala Leu Val Gly Pro Tyr Gln Asp Val Gln Ala Met Leu Ser Tyr Gly
                        485             490             495
  70    Gly Ser Thr Gln Leu Pro Lys Gly Cys Ser Ala Leu Ala Val Ser His
                    500             505             510
```

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```
         Gly Thr Asn Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Gly Lys
                 515             520             525

 5       Tyr Leu Pro Phe Glu Gly Ile Ala Leu Asp Asp Gln Gly Thr Leu Asn
                 530             535             540

         Leu Gln Phe Pro Asn Ala Thr Asp Lys Gln Lys Ala Ile Leu Gln Thr
         545             550             555             560
10
         Met Ser Asp Ile Ile Leu His Ile Arg Tyr Thr Ile Arg •••
                 565             570             573
```

15   (2) INFORMATION FOR SEQ ID NO:56

              (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 2994 base pairs
                   (B) TYPE: nucleic acid
20   (C) STRANDEDNESS: double
                   (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: DNA (genomic)

              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56 (tccA)
25
```
 1   ATG AAT CAA CTC GCC AGT CCC CTG ATT TCC CGC ACC GAA GAG ATC CAC   48
 1   Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His   16

30
49   AAC TTA CCC GGT AAA TTG ACC GAT CTT GGT TAT ACC TCA GTG TTT GAT   96
17   Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp   32


97   GTG GTA CGT ATG CCG CGT GAG CGT TTT ATT CGT GAG CAT CGT GCT GAT   144
35   33   Val Val Arg Met Pro Arg Glu Arg Phe Ile Arg Glu His Arg Ala Asp   48


145  CTC GGG CGC AGT GCT GAA AAA ATG TAT GAC CTG GCA GTG GGC TAT GCT   192
49   Leu Gly Arg Ser Ala Glu Lys Met Tyr Asp Leu Ala Val Gly Tyr Ala   64
40
193  CAT CAG GTG TTA CAC CAT TTT CGC CGT AAT TCT CTT AGT GAA GCT GTT   240
65   His Gln Val Leu His His Phe Arg Arg Asn Ser Leu Ser Glu Ala Val   80

45
241  CAG TTT GGC TTG AGA AGT CCG TTC TCC GTA TCA GGC CCG GAT TAC GCC   288
81   Gln Phe Gly Leu Arg Ser Pro Phe Ser Val Ser Gly Pro Asp Tyr Ala   96


289  AAT CAG TTT CTT GAT GCA AAC ACG GGT TGG AAA GAT AAA GCA CCA AGT   336
97   Asn Gln Phe Leu Asp Ala Asn Thr Gly Trp Lys Asp Lys Ala Pro Ser   112


337  GGA TCA CCG GAA GCC AAT GAT GCG CCG GTA GCC TAT CTG ACT CAT ATT   384
113  Gly Ser Pro Glu Ala Asn Asp Ala Pro Val Ala Tyr Leu Thr His Ile   128


385  TAT CAA TTG GCC CTT GAA CAG GAA AAG AAT GGC GCC ACT ACC ATT ATG   432
129  Tyr Gln Leu Ala Leu Glu Gln Glu Lys Asn Gly Ala Thr Thr Ile Met   144


433  AAT ACG CTG GCG GAG CGT CGC CCC GAT CTG GGT GCT TTG TTA ATT AAT   480
145  Asn Thr Leu Ala Glu Arg Arg Pro Asp Leu Gly Ala Leu Leu Ile Asn   160


481  GAT AAA GCA ATC AAT GAG GTG ATA CCG CAA TTG CAG TTG GTC AAT GAA   528
161  Asp Lys Ala Ile Asn Glu Val Ile Pro Gln Leu Gln Leu Val Asn Glu   176
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529   ATT CTG TCC AAA GCT ATT CAG AAG AAA CTG AGT TTG ACT GAT CTG GAA   576
177   Ile Leu Ser Lys Ala Ile Gln Lys Lys Leu Ser Leu Thr Asp Leu Glu   192


577   GCG GTA AAC GCC AGA CTT TCC ACT ACC CGT TAC CCG AAT AAT CTG CCG   624
193   Ala Val Asn Ala Arg Leu Ser Thr Thr Arg Tyr Pro Asn Asn Leu Pro   208


625   TAT CAT TAT GGT CAT CAG CAG ATT CAG ACA GCT CAA TCG GTA TTG GGT   672
209   Tyr His Tyr Gly His Gln Gln Ile Gln Thr Ala Gln Ser Val Leu Gly   224


673   ACT ACG TTG CAA GAT ATC ACT TTG CCA CAG ACG CTG GAT CTG CCG CAA   720
225   Thr Thr Leu Gln Asp Ile Thr Leu Pro Gln Thr Leu Asp Leu Pro Gln   240


721   AAC TTC TGG GCA ACA GCA AAA GGA AAA CTG AGC GAT ACG ACT GCC AGT   768
241   Asn Phe Trp Ala Thr Ala Lys Gly Lys Leu Ser Asp Thr Thr Ala Ser   256


769   GCT TTG ACC CGA CTG CAA ATC ATG GCG AGT CAG TTT TCG CCA GAG CAG   816
257   Ala Leu Thr Arg Leu Gln Ile Met Ala Ser Gln Phe Ser Pro Glu Gln   272


817   CAG AAA ATC ATT ACG GAG ACT GTC GGT CAG GAT TTC TAT CAG CTT AAC   864
273   Gln Lys Ile Ile Thr Glu Thr Val Gly Gln Asp Phe Tyr Gln Leu Asn   288


865   TAT GGT GAC AGT TCG CTT ACT GTG AAT AGT TTC AGC GAC ATG ACC ATA   912
289   Tyr Gly Asp Ser Ser Leu Thr Val Asn Ser Phe Ser Asp Met Thr Ile   304


913   ATG ACT GAT CGA ACA AGT TTG ACT GTA CCC CAG GTA GAA CTG ATG TTG   960
305   Met Thr Asp Arg Thr Ser Leu Thr Val Pro Gln Val Glu Leu Met Leu   320


961   TGT TCA ACT GTC GGA GGT TCT ACG GTT GTT AAG TCT GAT AAT GTG AGT   1008
1008
321   Cys Ser Thr Val Gly Gly Ser Thr Val Val Lys Ser Asp Asn Val Ser   336


1009  TCT GGT GAC ACG ACA GCG ACG CCA TTT GCG TAT GGC GCC CGC TTT ATT   1056
1056
337   Ser Gly Asp Thr Thr Ala Thr Pro Phe Ala Tyr Gly Ala Arg Phe Ile   352


1057  CAT GCC GGT AAG CCG GAG GCG ATT ACC CTG AGT CGC AGT GGT GCG GAG   1104
1104
353   His Ala Gly Lys Pro Glu Ala Ile Thr Leu Ser Arg Ser Gly Ala Glu   368


1105  GCG CAT TTT GCT CTG ACG GTT AAC AAT CTG ACA GAT GAC AAG TTG GAC   1152
1152
369   Ala His Phe Ala Leu Thr Val Asn Asn Leu Thr Asp Asp Lys Leu Asp   384


1153  CGT ATT AAC CGC ACA GTG CGC CTG CAA AAA TGG CTG AAT CTG CCT TAT   1200
1200
385   Arg Ile Asn Arg Thr Val Arg Leu Gln Lys Trp Leu Asn Leu Pro Tyr   400


1201  GAG GAT ATT GAC CTG TTA GTG ACT TCT GCT ATG GAT GCG GAA ACA GGA   1248
1248
401   Glu Asp Ile Asp Leu Leu Val Thr Ser Ala Met Asp Ala Glu Thr Gly   416


1249  AAT ACC GCG CTG TCG ATG AAC GAC AAT ACG CTG CGT ATG TTG GGA GTG   1296
1296
417   Asn Thr Ala Leu Ser Met Asn Asp Asn Thr Leu Arg Met Leu Gly Val   432
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1297   TTC AAA CAT TAT CAG GCG AAG TAT GGT GTT AGC GCT AAA CAA TTT GCT
1344
433   Phe Lys His Tyr Gln Ala Lys Tyr Gly Val Ser Ala Lys Gln Phe Ala   448

1345   GGC TGG CTG CGC GTA GTG GCC CCG TTT GCC ATT ACA CCG GCA ACG CCG
1392
449   Gly Trp Leu Arg Val Val Ala Pro Phe Ala Ile Thr Pro Ala Thr Pro   464

1393   TTT TTA GAC CAA GTG TTT AAC TCC GTC GGC ACC TTT GAT ACA CCG_TTT
1440
465   Phe Leu Asp Gln Val Phe Asn Ser Val Gly Thr Phe Asp Thr Pro Phe   480

1441   GTG ATA GAT AAT CAG GAT TTT GTC TAT ACA TTG ACC ACC GGG GGC GAT
1488
481   Val Ile Asp Asn Gln Asp Phe Val Tyr Thr Leu Thr Thr Gly Gly Asp   496

1489   GGG GCG CGT GTT AAG CAT ATC AGC ACG GCA CTG GGC CTC AAT CAT CGT
1536
497   Gly Ala Arg Val Lys His Ile Ser Thr Ala Leu Gly Leu Asn His Arg   512

1537   CAG TTC CTG TTA TTG GCG GAT AAT ATT GCC CGT CAA CAG GGG AAT GTC
1584
513   Gln Phe Leu Leu Leu Ala Asp Asn Ile Ala Arg Gln Gln Gly Asn Val   528

1585   ACG CAA AGC ACA CTC AAC TGT AAT CTG TTT GTG GTG TCA GCT TTC TAC
1632
529   Thr Gln Ser Thr Leu Asn Cys Asn Leu Phe Val Val Ser Ala Phe Tyr   544

1633   CGT CTG GCT AAT TTG GCG CGC ACA TTG GGG ATA AAT CCA GAG TCT TTC
1680
545   Arg Leu Ala Asn Leu Ala Arg Thr Leu Gly Ile Asn Pro Glu Ser Phe   560

1681   TGT GCC TTG GTT GAT CGA TTA GAT GCA GGT ACA GGC ATC GTC TGG CAG
1728
561   Cys Ala Leu Val Asp Arg Leu Asp Ala Gly Thr Gly Ile Val Trp Gln   576

1729   CAA TTG GCA GGG AAA CCC ACA ATC ACG GTA CCA CAA AAA GAT TCC CCG
1776
577   Gln Leu Ala Gly Lys Pro Thr Ile Thr Val Pro Gln Lys Asp Ser Pro   592

1777   CTG GCG GCG GAT ATT CTG AGT TTG CTG CAA GCG CTA AGT GCG ATT GCT
1824
593   Leu Ala Ala Asp Ile Leu Ser Leu Leu Gln Ala Leu Ser Ala Ile Ala   608

1825   CAA TGG CAA CAA CAG CAC GAT TTA GAA TTT TCA GCA CTG CTT TTG CTG
1872
609   Gln Trp Gln Gln Gln His Asp Leu Glu Phe Ser Ala Leu Leu Leu Leu   624

1873   TTG AGT GAC AAC CCT ATT TCT ACC TCG CAG GGC ACT GAC GAT CAA TTG
1920
625   Leu Ser Asp Asn Pro Ile Ser Thr Ser Gln Gly Thr Asp Asp Gln Leu   640

1921   AAC TTT ATC CGT CAA GTG TGG CAG AAC CTA GGC AGT ACG TTT GTG GGT
1968
641   Asn Phe Ile Arg Gln Val Trp Gln Asn Leu Gly Ser Thr Phe Val Gly   656

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SUBSTITUTE SHEET (RULE 26)

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1969  GCA ACA TTG TTG TCC CGC AGT GGG GCA CCA TTA GTC GAT ACC AAC GGC
2016
 657  Ala Thr Leu Leu Ser Arg Ser Gly Ala Pro Leu Val Asp Thr Asn Gly  672

2017  CAC GCT ATT GAC TGG TTT GCT CTG CTC TCA GCA GGT AAT AGT CCG CTT
2064
 673  His Ala Ile Asp Trp Phe Ala Leu Leu Ser Ala Gly Asn Ser Pro Leu  688

2065  ATC GAT AAG GTT GGT CTG GTG ACT GAT GCT GGC ATA CAA AGT GTT ATA
2112
 689  Ile Asp Lys Val Gly Leu Val Thr Asp Ala Gly Ile Gln Ser Val Ile  704

2113  GCA ACG GTG GTC AAT ACA CAA AGC TTA TCT GAT GAA GAT AAG AAG CTG
2160
 705  Ala Thr Val Val Asn Thr Gln Ser Leu Ser Asp Glu Asp Lys Lys Leu  720

2161  GCA ATC ACT ACT CTG ACT AAT ACG TTG AAT CAG GTA CAG AAA ACT CAA
2208
 721  Ala Ile Thr Thr Leu Thr Asn Thr Leu Asn Gln Val Gln Lys Thr Gln  736

2209  CAG GGC GTG GCC GTC AGT CTG TTG GCG CAG ACT CTG AAC GTG AGT CAG
2256
 737  Gln Gly Val Ala Val Ser Leu Leu Ala Gln Thr Leu Asn Val Ser Gln  752

2257  TCA CTG CCT GCG TTA TTG TTG CGC TGG AGT GGA CAA ACA ACC TAC CAG
2304
 753  Ser Leu Pro Ala Leu Leu Leu Arg Trp Ser Gly Gln Thr Thr Tyr Gln  768

2305  TGG TTG AGT GCG ACT TGG GCA TTG AAG GAT GCC GTT AAG ACT GCC GCC
2352
 769  Trp Leu Ser Ala Thr Trp Ala Leu Lys Asp Ala Val Lys Thr Ala Ala  784

2353  GAT ATT CCC GCT GAC TAT CTG CGT CAA TTA CGT GAA GTG GTA CGC CGC
2400
 785  Asp Ile Pro Ala Asp Tyr Leu Arg Gln Leu Arg Glu Val Val Arg Arg  800

2401  TCC TTG TTG ACC CAA CAA TTC ACG CTG AGT CCT GCA ATG GTG CAA ACC
2448
 801  Ser Leu Leu Thr Gln Gln Phe Thr Leu Ser Pro Ala Met Val Gln Thr  816

2449  TTG CTG GAC TAT CCA GCC TAT TTT GGC GCT TCC GCA GAA ACA GTG ACC
2496
 817  Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr  832

2497  GAT ATC AGT TTG TGG ATG CTT TAT ACC CTG AGC TGT TAT AGC GAT TTA
2544
 833  Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu  848

2545  TTG CTC CAA ATG GGT GAA GCT GGT GGT ACC GAA GAT GAT GTA CTG GCC
2592
 849  Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala  864

2593  TAC TTA CGC ACA GCT AAT GCT ACC ACA CCG TTG AGC CAA TCT GAT GCT
2640
 865  Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala  880
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2641  GCA CAG ACG TTG GCA ACG CTA TTG GGT TGG GAG GTT AAC GAG TTG CAA
2688
 881  Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln  896

2689  GCC GCT TGG TCG GTA TTG GGC GGG ATT GCC AAA ACC ACA CCG CAA CTG
2736
 897  Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu  912

2737  GAT GCG CTT CTG CGT TTG CAA CAG GCA CAG AAC CAA ACT GGT CTT GGC
2784
 913  Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly  928

2785  GTT ACA CAG CAA CAG CAA GGC TAT CTC CTG AGT CGT GAC AGT GAT TAT
2832
 929  Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr  944

2833  ACC CTT TGG CAA AGC ACC GGT CAG GCG CTG GTG GCT GGC GTA TCC CAT
2880
 945  Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His  960

2881  GTC AAG GGC AGT AAC TGA GCATGGCAGA GCTCACTACC TGAGTGGATT TGATTT
2934
 961  Val Lys Gly Ser Asn End                                          965

2935  TTCCGTATGG CCTAATGAGG CTATTTCTAA ACCGCCATTT AAGTAAGGCA GATAATTATG
2994
```

(2) INFORMATION FOR SEQ ID NO:57

        (i)   SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 965 amino acids
              (B) TYPE: amino acid
              (C) TOPOLOGY: linear
       (ii)   MOLECULE TYPE: protein

       (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:57 (TccA peptide)
              Features   From   To   Description
                             1   10   SEQ ID NO:8

```
   1  Met Asn Gln Leu Ala Ser Pro Leu Ile Ser Arg Thr Glu Glu Ile His   16

  17  Asn Leu Pro Gly Lys Leu Thr Asp Leu Gly Tyr Thr Ser Val Phe Asp   32

  33  Val Val Arg Met Pro Arg Glu Arg Phe Ile Arg Glu His Arg Ala Asp   48

  49  Leu Gly Arg Ser Ala Glu Lys Met Tyr Asp Leu Ala Val Gly Tyr Ala   64

  65  His Gln Val Leu His His Phe Arg Arg Asn Ser Leu Ser Glu Ala Val   80

  81  Gln Phe Gly Leu Arg Ser Pro Phe Ser Val Ser Gly Pro Asp Tyr Ala   96

  97  Asn Gln Phe Leu Asp Ala Asn Thr Gly Trp Lys Asp Lys Ala Pro Ser  112

 113  Gly Ser Pro Glu Ala Asn Asp Ala Pro Val Ala Tyr Leu Thr His Ile  128

 129  Tyr Gln Leu Ala Leu Glu Gln Glu Lys Asn Gly Ala Thr Thr Ile Met  144

 145  Asn Thr Leu Ala Glu Arg Arg Pro Asp Leu Gly Ala Leu Leu Ile Asn  160

 161  Asp Lys Ala Ile Asn Glu Val Ile Pro Gln Leu Gln Leu Val Asn Glu  176

 177  Ile Leu Ser Lys Ala Ile Gln Lys Lys Leu Ser Leu Thr Asp Leu Glu  192
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	193	Ala	Val	Asn	Ala	Arg	Leu	Ser	Thr	Thr	Arg	Tyr	Pro	Asn	Asn	Leu	Pro	208
5	209	Tyr	His	Tyr	Gly	His	Gln	Gln	Ile	Gln	Thr	Ala	Gln	Ser	Val	Leu	Gly	224
	225	Thr	Thr	Leu	Gln	Asp	Ile	Thr	Leu	Pro	Gln	Thr	Leu	Asp	Leu	Pro	Gln	240
	241	Asn	Phe	Trp	Ala	Thr	Ala	Lys	Gly	Lys	Leu	Ser	Asp	Thr	Thr	Ala	Ser	256
10	257	Ala	Leu	Thr	Arg	Leu	Gln	Ile	Met	Ala	Ser	Gln	Phe	Ser	Pro	Glu	Gln	272
	273	Gln	Lys	Ile	Ile	Thr	Glu	Thr	Val	Gly	Gln	Asp	Phe	Tyr	Gln	Leu	Asn	288
15	289	Tyr	Gly	Asp	Ser	Ser	Leu	Thr	Val	Asn	Ser	Phe	Ser	Asp	Met	Thr	Ile	304
	305	Met	Thr	Asp	Arg	Thr	Ser	Leu	Thr	Val	Pro	Gln	Val	Glu	Leu	Met	Leu	320
	321	Cys	Ser	Thr	Val	Gly	Gly	Ser	Thr	Val	Val	Lys	Ser	Asp	Asn	Val	Ser	336
20	337	Ser	Gly	Asp	Thr	Thr	Ala	Thr	Pro	Phe	Ala	Tyr	Gly	Ala	Arg	Phe	Ile	352
	353	His	Ala	Gly	Lys	Pro	Glu	Ala	Ile	Thr	Leu	Ser	Arg	Ser	Gly	Ala	Glu	368
25	369	Ala	His	Phe	Ala	Leu	Thr	Val	Asn	Asn	Leu	Thr	Asp	Asp	Lys	Leu	Asp	384
	385	Arg	Ile	Asn	Arg	Thr	Val	Arg	Leu	Gln	Lys	Trp	Leu	Asn	Leu	Pro	Tyr	400
	401	Glu	Asp	Ile	Asp	Leu	Leu	Val	Thr	Ser	Ala	Met	Asp	Ala	Glu	Thr	Gly	416
30	417	Asn	Thr	Ala	Leu	Ser	Met	Asn	Asp	Asn	Thr	Leu	Arg	Met	Leu	Gly	Val	432
	433	Phe	Lys	His	Tyr	Gln	Ala	Lys	Tyr	Gly	Val	Ser	Ala	Lys	Gln	Phe	Ala	448
35	449	Gly	Trp	Leu	Arg	Val	Val	Ala	Pro	Phe	Ala	Ile	Thr	Pro	Ala	Thr	Pro	464
	465	Phe	Leu	Asp	Gln	Val	Phe	Asn	Ser	Val	Gly	Thr	Phe	Asp	Thr	Pro	Phe	480
	481	Val	Ile	Asp	Asn	Gln	Asp	Phe	Val	Tyr	Thr	Leu	Thr	Thr	Gly	Gly	Asp	496
40	497	Gly	Ala	Arg	Val	Lys	His	Ile	Ser	Thr	Ala	Leu	Gly	Leu	Asn	His	Arg	512
	513	Gln	Phe	Leu	Leu	Leu	Ala	Asp	Asn	Ile	Ala	Arg	Gln	Gln	Gly	Asn	Val	528
45	529	Thr	Gln	Ser	Thr	Leu	Asn	Cys	Asn	Leu	Phe	Val	Val	Ser	Ala	Phe	Tyr	544
	545	Arg	Leu	Ala	Asn	Leu	Ala	Arg	Thr	Leu	Gly	Ile	Asn	Pro	Glu	Ser	Phe	560
	561	Cys	Ala	Leu	Val	Asp	Arg	Leu	Asp	Ala	Gly	Thr	Gly	Ile	Val	Trp	Gln	576
50	577	Gln	Leu	Ala	Gly	Lys	Pro	Thr	Ile	Thr	Val	Pro	Gln	Lys	Asp	Ser	Pro	592
	593	Leu	Ala	Ala	Asp	Ile	Leu	Ser	Leu	Leu	Gln	Ala	Leu	Ser	Ala	Ile	Ala	608
55	609	Gln	Trp	Gln	Gln	Gln	His	Asp	Leu	Glu	Phe	Ser	Ala	Leu	Leu	Leu	Leu	624
	625	Leu	Ser	Asp	Asn	Pro	Ile	Ser	Thr	Ser	Gln	Gly	Thr	Asp	Asp	Gln	Leu	640
	641	Asn	Phe_Ile	Arg	Gln	Val	Trp	Gln	Asn	Leu	Gly	Ser	Thr	Phe	Val	Gly	656
60	657	Ala	Thr	Leu	Leu	Ser	Arg	Ser	Gly	Ala	Pro	Leu	Val	Asp	Thr	Asn	Gly	672
	673	His	Ala	Ile	Asp	Trp	Phe	Ala	Leu	Leu	Ser	Ala	Gly	Asn	Ser	Pro	Leu	688
65	689	Ile	Asp	Lys	Val	Gly	Leu	Val	Thr	Asp	Ala	Gly	Ile	Gln	Ser	Val	Ile	704
	705	Ala	Thr	Val	Val	Asn	Thr	Gln	Ser	Leu	Ser	Asp	Glu	Asp	Lys	Lys	Leu	720
	721	Ala	Ile	Thr	Thr	Leu	Thr	Asn	Thr	Leu	Asn	Gln	Val	Gln	Lys	Thr	Gln	736
70	737	Gln	Gly	Val	Ala	Val	Ser	Leu	Leu	Ala	Gln	Thr	Leu	Asn	Val	Ser	Gln	752

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    753  Ser Leu Pro Ala Leu Leu Leu Arg Trp Ser Gly Gln Thr Thr Tyr Gln  768

    769  Trp Leu Ser Ala Thr Trp Ala Leu Lys Asp Ala Val Lys Thr Ala Ala  784

5   785  Asp Ile Pro Ala Asp Tyr Leu Arg Gln Leu Arg Glu Val Val Arg Arg  800

    801  Ser Leu Leu Thr Gln Gln Phe Thr Leu Ser Pro Ala Met Val Gln Thr  816

    817  Leu Leu Asp Tyr Pro Ala Tyr Phe Gly Ala Ser Ala Glu Thr Val Thr  832
10
    833  Asp Ile Ser Leu Trp Met Leu Tyr Thr Leu Ser Cys Tyr Ser Asp Leu  848

    849  Leu Leu Gln Met Gly Glu Ala Gly Gly Thr Glu Asp Asp Val Leu Ala  864
15
    865  Tyr Leu Arg Thr Ala Asn Ala Thr Thr Pro Leu Ser Gln Ser Asp Ala  880

    881  Ala Gln Thr Leu Ala Thr Leu Leu Gly Trp Glu Val Asn Glu Leu Gln  896
20
    897  Ala Ala Trp Ser Val Leu Gly Gly Ile Ala Lys Thr Thr Pro Gln Leu  912

    913  Asp Ala Leu Leu Arg Leu Gln Gln Ala Gln Asn Gln Thr Gly Leu Gly  928

    929  Val Thr Gln Gln Gln Gln Gly Tyr Leu Leu Ser Arg Asp Ser Asp Tyr  944
25
    945  Thr Leu Trp Gln Ser Thr Gly Gln Ala Leu Val Ala Gly Val Ser His  960

    961  Val Lys Gly Ser Asn   965
```

30 (2) INFORMATION FOR SEQ ID NO:58

         (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 4932 base pairs
              (B) TYPE: nucleic acid
35   (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: DNA (genomic)

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58 (tccB)
40
```
      1  ATG TTA TCG ACA ATG GAA AAA CAA CTG AAT GAA TCC CAG CGT GAT GCG   48
      1  Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp Ala   16
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45    49  TTG GTG ACT GGC TAT ATG AAT TTT GTG GCG CCG ACG TTG AAA GGC GTC   96
      17  Leu Val Thr Gly Tyr Met Asn Phe Val Ala Pro Thr Leu Lys Gly Val   32
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      97  AGT GGT CAG CCG GTG ACG GTG GAA GAT TTA TAC GAA TAT TTG CTG ATT  144
50    33  Ser Gly Gln Pro Val Thr Val Glu Asp Leu Tyr Glu Tyr Leu Leu Ile   48
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     145  GAC CCG GAA GTG GCT GAT GAG GTT GAG ACG AGT CGG GTA GCA CAA GCG  192
      49  Asp Pro Glu Val Ala Asp Glu Val Glu Thr Ser Arg Val Ala Gln Ala   64
55
     193  ATT GCC AGC ATA CAG CAA TAT ATG ACT CGT CTG GTC AAC GGC TCT GAA  240
      65  Ile Ala Ser Ile Gln Gln Tyr Met Thr Arg Leu Val Asn Gly Ser Glu   80
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```
     241  CCG GGG CGT CAG GCG ATG GAG CCT TCT ACA GCT AAC GAA TGG CGT GAT  288
60    81  Pro Gly Arg Gln Ala Met Glu Pro Ser Thr Ala Asn Glu Trp Arg Asp   96
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     289  AAT GAT AAC CAA TAT GCT ATC TGG GCT GCG GGG GCT GAG GTT CGA AAT  336
      97  Asn Asp Asn Gln Tyr Ala Ile Trp Ala Ala Gly Ala Glu Val Arg Asn  112
65
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     337  TAC GCT GAA AAC TAT ATT TCA CCC ATC ACC CGG CAG GAA AAA AGC CAT  384
     113  Tyr Ala Glu Asn Tyr Ile Ser Pro Ile Thr Arg Gln Glu Lys Ser His  128
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        385  TAT TTC TCG GAG CTG GAG ACG ACT TTA AAT CAG AAT CGA CTC GAT CCG  432
        129  Tyr Phe Ser Glu Leu Glu Thr Thr Leu Asn Gln Asn Arg Leu Asp Pro  144

  5     433  GAT CGT GTG CAG GAT GCT GTT TTG GCG TAT CTC AAT GAG TTT GAG GCA  480
        145  Asp Arg Val Gln Asp Ala Val Leu Ala Tyr Leu Asn Glu Phe Glu Ala  160

        481  GTG AGT AAT CTA TAT GTG CTC AGT GGT TAT ATT AAT CAG GAT AAA TTT  528
 10     161  Val Ser Asn Leu Tyr Val Leu Ser Gly Tyr Ile Asn Gln Asp Lys Phe  176

        529  GAC CAA GCT ATC TAC TAC TTT ATT GGT CGC ACT ACC ACT AAA CCG TAT  576
        177  Asp Gln Ala Ile Tyr Tyr Phe Ile Gly Arg Thr Thr Thr Lys Pro Tyr  192
 15
        577  CGC TAC TAC TGG CGT CAG ATG GAT TTG AGT AAG AAC CGT CAA GAT CCG  624
        193  Arg Tyr Tyr Trp Arg Gln Met Asp Leu Ser Lys Asn Arg Gln Asp Pro  208
 20
        625  GCA GGG AAT CCG GTG ACG CCA AAT TGC TGG AAT GAT TGG CAG GAA ATC  672
        209  Ala Gly Asn Pro Val Thr Pro Asn Cys Trp Asn Asp Trp Gln Glu Ile  224

 25     673  ACT TTG CCG CTG TCT GGT GAT ACG GTG CTG GAG CAT ACA GTT CGC CCG  720
        225  Thr Leu Pro Leu Ser Gly Asp Thr Val Leu Glu His Thr Val Arg Pro  240

        721  GTA TTT TAT AAT GAT CGA CTA TAT GTG GCT TGG GTT GAG CGT GAC CCG  768
 30     241  Val Phe Tyr Asn Asp Arg Leu Tyr Val Ala Trp Val Glu Arg Asp Pro  256

        769  GCA GTA CAG AAG GAT GCT GAC GGT AAA AAC ATC GGT AAA ACC CAT GCC  816
        257  Ala Val Gln Lys Asp Ala Asp Gly Lys Asn Ile Gly Lys Thr His Ala  272
 35
        817  TAC AAC ATA AAG TTT GGT TAT AAA CGT TAT GAT GAT ACT TGG ACA GCG  864
        273  Tyr Asn Ile Lys Phe Gly Tyr Lys Arg Tyr Asp Asp Thr Trp Thr Ala  288
 40
        865  CCG AAT ACG ACC ACG TTA ATG ACA CAA CAA GCA GGG GAA AGT TCA GAA  912
        289  Pro Asn Thr Thr Thr Leu Met Thr Gln Gln Ala Gly Glu Ser Ser Glu  304

 45     913  ACA CAG CGA TCC AGC CTG CTG ATT GAT GAA TCT AGC ACC ACA TTG CGC  960
        305  Thr Gln Arg Ser Ser Leu Leu Ile Asp Glu Ser Ser Thr Thr Leu Arg  320

        961  CAA GTT AAT CTG TTG GCT ACC ACC GAT TTT AGT ATC GAT CCG ACG GAG
 50    1008
        321  Gln Val Asn Leu Leu Ala Thr Thr Asp Phe Ser Ile Asp Pro Thr Glu  336

       1009  GAA ACG GAC AGT AAC CCG TAT GGC CGC CTA ATG TTG GGG GTG TTT GTC
 55    1056
        337  Glu Thr Asp Ser Asn Pro Tyr Gly Arg Leu Met Leu Gly Val Phe Val  352

       1057  CGT CAA TTT GAA GGT GAT GGG GCC AAT AGA AAA AAT AAA CCC GTT GTT
 60    1104
        353  Arg Gln Phe Glu Gly Asp Gly Ala Asn Arg Lys Asn Lys Pro Val Val  368

       1105  TAT GGT TAT CTC TAT TGT GAC TCA GCT TTC AAT CGT CAT GTT CTC AGG
 65    1152
        369  Tyr Gly Tyr Leu Tyr Cys Asp Ser Ala Phe Asn Arg His Val Leu Arg  384

       1153  CCG TTA AGT AAG AAC TTT TTG TTC AGT ACT TAC CGT GAT GAA ACG GAT
 70    1200
        385  Pro Leu Ser Lys Asn Phe Leu Phe Ser Thr Tyr Arg Asp Glu Thr Asp  400
```

SUBSTITUTE SHEET (RULE 26)

```
      1201  GGT CAA AAC AGC TTG CAA TTT GCG GTA TAC GAT AAA AAG TAT GTA ATT
      1248
 5     401  Gly Gln Asn Ser Leu Gln Phe Ala Val Tyr Asp Lys Lys Tyr Val Ile  416


      1249  ACT AAG GTT GTT ACA GGT GCA ACG GAA GAT CCC GAA AAT ACA GGA TGG
      1296
10     417  Thr Lys Val Val Thr Gly Ala Thr Glu Asp Pro Glu Asn Thr Gly Trp  432


      1297  GTA AGT AAA GTT GAT GAC TTG AAA CAA GGC ACT ACT GGG GCC TAT GTG
      1344
15     433  Val Ser Lys Val Asp Asp Leu Lys Gln Gly Thr Thr Gly Ala Tyr Val  448


      1345  TAT ATC GAT CAA GAT GGC CTG ACG CTT CAT ATA CAA ACC ACA ACT AAT
      1392
20     449  Tyr Ile Asp Gln Asp Gly Leu Thr Leu His Ile Gln Thr Thr Thr Asn  464


      1393  GGG GAT TTT ATT AAC CGT CAT ACG TTT GGA TAT AAC GAT CTT GTA TAT
      1440
25     465  Gly Asp Phe Ile Asn Arg His Thr Phe Gly Tyr Asn Asp Leu Val Tyr  480


      1441  GAT TCT AAG TCT GGT TAT GGT TTC ACG TGG TCA GGA AAT GAA GGT TTT
      1488
30     481  Asp Ser Lys Ser Gly Tyr Gly Phe Thr Trp Ser Gly Asn Glu Gly Phe  496


      1489  TAT CTG GAT TAC CAT GAT GGA AAT TAT TAC ACC TTT CAT AAT GCA ATA
      1536
35     497  Tyr Leu Asp Tyr His Asp Gly Asn Tyr Tyr Thr Phe His Asn Ala Ile  512


      1537  ATC AAC TAC TAT CCG TCT GGA TAT GGT GGT GGA TCT GTT CCT AAT GGA
      1584
40     513  Ile Asn Tyr Tyr Pro Ser Gly Tyr Gly Gly Gly Ser Val Pro Asn Gly  528


      1585  ACG TGG GCG TTA GAG CAA AGG ATT AAT GAG GGA TGG GCT ATT GCT CCC
      1632
45     529  Thr Trp Ala Leu Glu Gln Arg Ile Asn Glu Gly Trp Ala Ile Ala Pro  544


      1633  CTG CTT GAT ACT CTC CAT ACT GTT ACT GTG AAG GGC AGT TAT ATC GCT
      1680
50     545  Leu Leu Asp Thr Leu His Thr Val Thr Val Lys Gly Ser Tyr Ile Ala  560


      1681  TGG GAA GGG GAA ACA CCT ACC GGT TAT AAT CTG TAT ATT CCA GAT GGT
      1728
55     561  Trp Glu Gly Glu Thr Pro Thr Gly Tyr Asn Leu Tyr Ile Pro Asp Gly  576


      1729  ACC GTG TTG CTA GAT TGG TTT GAT AAA ATA AAT TTT GCT ATT GGT CTT
      1776
60     577  Thr Val Leu Leu Asp Trp Phe Asp Lys Ile Asn Phe Ala Ile Gly Leu  592


      1777  AAT AAG CTT GAG TCT GTA TTT ACG TCG CCA GAT TGG CCA ACA CTA ACC
      1824
65     593  Asn Lys Leu Glu Ser Val Phe Thr Ser Pro Asp Trp Pro Thr Leu Thr  608


      1825  ACT ATC AAA AAT TTC AGT AAA ATC GCC GAT AAC CGC AAA TTC TAT CAG
      1872
70     609  Thr Ile Lys Asn Phe Ser Lys Ile Ala Asp Asn Arg Lys Phe Tyr Gln  624
```

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SUBSTITUTE SHEET (RULE 26)

```
       1873  GAA ATC AAT GCT GAG ACG GCG GAT GGA CGC AAC CTG TTT AAA CGT TAC
       1920
        625  Glu Ile Asn Ala Glu Thr Ala Asp Gly Arg Asn Leu Phe Lys Arg Tyr  640

       1921  AGT ACT CAA ACT TTC GGA CTT ACC AGC GGT GCG ACT TAT TCT ACA ACT
       1968
        641  Ser Thr Gln Thr Phe Gly Leu Thr Ser Gly Ala Thr Tyr Ser Thr Thr  656

       1969  TAT ACT TTG TCT GAG GCG GAT TTC TCC ACT GAT CCG GAC AAA AAC TAC
       2016
        657  Tyr Thr Leu Ser Glu Ala Asp Phe Ser Thr Asp Pro Asp Lys Asn Tyr  672

       2017  CTA CAG GTT TGT TTG AAT GTC GTG TGG GAT CAT TAT GAC CGC CCG TCA
       2064
        673  Leu Gln Val Cys Leu Asn Val Val Trp Asp His Tyr Asp Arg Pro Ser  688

       2065  GGG AAA AAA GGG GCT TAT TCT TGG GTC AGT AAG TGG TTT AAC GTC TAT
       2112
        689  Gly Lys Lys Gly Ala Tyr Ser Trp Val Ser Lys Trp Phe Asn Val Tyr  704

       2113  GTT GCG TTG CAA GAT AGC AAA GCT CCG GAT GCC ATT CCT CGA TTA GTT
       2160
        705  Val Ala Leu Gln Asp Ser Lys Ala Pro Asp Ala Ile Pro Arg Leu Val  720

       2161  TCC CGT TAC GAT AGT AAA CGT GGT CTG GTG CAA TAT CTG GAC TTC TGG
       2208
        721  Ser Arg Tyr Asp Ser Lys Arg Gly Leu Val Gln Tyr Leu Asp Phe Trp  736

       2209  ACC TCA TCA TTA CCC GCG AAA ACC CGT CTT AAC ACC ACC TTT GTG CGT
       2256
        737  Thr Ser Ser Leu Pro Ala Lys Thr Arg Leu Asn Thr Thr Phe Val Arg  752

       2257  ACT TTG ATT GAG AAG GCT AAT CTG GGG CTG GAT AGT TTG CTG GAT TAC
       2304
        753  Thr Leu Ile Glu Lys Ala Asn Leu Gly Leu Asp Ser Leu Leu Asp Tyr  768

       2305  ACC TTG CAG GCA GAT CCT TCT CTG GAA GCA GAT TTA GTG ACT GAC GGC
       2352
        769  Thr Leu Gln Ala Asp Pro Ser Leu Glu Ala Asp Leu Val Thr Asp Gly  784

       2353  AAA AGC GAA CCA ATG GAC TTT AAT GGT TCA AAC GGT CTC TAT TTC TGG
       2400
        785  Lys Ser Glu Pro Met Asp Phe Asn Gly Ser Asn Gly Leu Tyr Phe Trp  800

       2401  GAA TTG TTC TTT CAC CTG CCG TTT TTG GTT GCT ACA CGC TTT GCC AAC
       2448
        801  Glu Leu Phe Phe His Leu Pro Phe Leu Val Ala Thr Arg Phe Ala Asn  816

       2449  GAA CAG CAA TTT TCG CCG GCA CAA AAG AGT TTG CAT TAC ATC TTT GAC
       2496
        817  Glu Gln Gln Phe Ser Pro Ala Gln Lys Ser Leu His Tyr Ile Phe Asp  832

       2497  CCG GCG ATG AAA AAC AAG CCA CAC AAT GCC CCG GCT TAT TGG AAT GTA
       2544
        833  Pro Ala Met Lys Asn Lys Pro His Asn Ala Pro Ala Tyr Trp Asn Val  848
```

SUBSTITUTE SHEET (RULE 26)

```
2545  CGT CCG TTG GTT GAA GGA AAC AGC GAT TTG TCA CGT CAT TTG GAC GAT
2592
 849  Arg Pro Leu Val Glu Gly Asn Ser Asp Leu Ser Arg His Leu Asp Asp  864
```

```
2593  TCT ATA GAC CCA GAT ACT CAA GCT TAT GCT CAT CCG GTG ATA TAC CAG
2640
 865  Ser Ile Asp Pro Asp Thr Gln Ala Tyr Ala His Pro Val Ile Tyr Gln  880
```

```
2641  AAA GCG GTG TTT ATT GCC TAT GTC AGT AAC CTG ATT GCT CAG GGA GAT
2688
 881  Lys Ala Val Phe Ile Ala Tyr Val Ser Asn Leu Ile Ala Gln Gly Asp  896
```

```
2689  ATG TGG TAT CGC CAA TTG ACT CGT GAC GGT CTG ACT CAG GCC CGT GTC
2736
 897  Met Trp Tyr Arg Gln Leu Thr Arg Asp Gly Leu Thr Gln Ala Arg Val  912
```

```
2737  TAT TAC AAT CTG GCC GCT GAA TTG CTA GGG CCT CGT CCG GAT GTA TCG
2784
 913  Tyr Tyr Asn Leu Ala Ala Glu Leu Leu Gly Pro Arg Pro Asp Val Ser  928
```

```
2785  CTG AGT AGC ATT TGG ACG CCG CAA ACC CTG GAT ACC TTA GCA GCC GGG
2832
 929  Leu Ser Ser Ile Trp Thr Pro Gln Thr Leu Asp Thr Leu Ala Ala Gly  944
```

```
2833  CAA AAA GCG GTT TTA CGT GAT TTT GAG CAC CAG TTG GCT AAT AGT GAT
2880
 945  Gln Lys Ala Val Leu Arg Asp Phe Glu His Gln Leu Ala Asn Ser Asp  960
```

```
2881  ACC GCT TTA CCC GCA TTG CCG GGC CGC AAT GTC AGC TAC TTG AAA CTG
2928
 961  Thr Ala Leu Pro Ala Leu Pro Gly Arg Asn Val Ser Tyr Leu Lys Leu  976
```

```
2929  GCA GAT AAT GGC TAC TTT AAT GAA CCG CTC AAT GTT CTG ATG TTG TCT
2976
 977  Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val Leu Met Leu Ser  992
```

```
2977  CAC TGG GAT ACG TTG GAT GCA CGG TTA TAC AAT CTG CGT CAT AAC CTG
3024
 993  His Trp Asp Thr Leu Asp Ala Arg Leu Tyr Asn Leu Arg His Asn Leu  1008
```

```
3025  ACC GTT GAT GGC AAG CCG CTT TCG CTG CCG CTG TAT GCT GCG CCT GTT
3072
1009  Thr Val Asp Gly Lys Pro Leu Ser Leu Pro Leu Tyr Ala Ala Pro Val  1024
```

```
3073  GAT CCG GTA GCG TTG TTG GCT CAG CGT GCT CAG TCC GGC ACG TTG ACG
3120
1025  Asp Pro Val Ala Leu Leu Ala Gln Arg Ala Gln Ser Gly Thr Leu Thr  1040
```

```
3121  AAT GGC GTC AGT GGC GCC ATG TTG ACG GTG CCG CCA TAC CGT TTC AGC
3168
1041  Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser  1056
```

```
3169  GCT ATG TTG CCG CGA GCT TAC AGC GCC GTG GGT ACG TTG ACC AGT TTT
3216
```

SUBSTITUTE SHEET (RULE 26)

```
      1057  Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe
      1072

 5    3217  GGT CAG AAC CTG CTT AGT TTG TTG GAA CGT AGC GAA CGA GCC TGT CAA
      3264
      1073  Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln
      1088

10
      3265  GAA GAG TTG GCG CAA CAG CAA CTG TTG GAT ATG TCC AGC TAT GCC ATC
      3312
      1089  Glu Glu Leu Ala Gln Gln Gln Leu Leu Asp Met Ser Ser Tyr Ala Ile
      1104

15
      3313  ACG TTG CAA CAA CAG GCG CTG GAT GGA TTG GCG GCA GAT CGT CTG GCG
      3360
      1105  Thr Leu Gln Gln Gln Ala Leu Asp Gly Leu Ala Ala Asp Arg Leu Ala
20    1120

      3361  CTG CTA GCT AGT CAG GCT ACG GCA CAA CAG CGT CAT GAC CAT TAT TAC
      3408
25    1121  Leu Leu Ala Ser Gln Ala Thr Ala Gln Gln Arg His Asp His Tyr Tyr
      1136

      3409  ACT CTG TAT CAG AAC AAC ATC TCC AGT GCG GAA CAA CTG GTG ATG GAC
30    3456
      1137  Thr Leu Tyr Gln Asn Asn Ile Ser Ser Ala Glu Gln Leu Val Met Asp
      1152

35    3457  ACC CAA ACG TCA GCA CAA TCC CTG ATT TCT TCT TCC ACT GGT GTA CAA
      3504
      1153  Thr Gln Thr Ser Ala Gln Ser Leu Ile Ser Ser Ser Thr Gly Val Gln
      1168

40
      3505  ACT GCC AGT GGG GCA CTG AAA GTG ATC CCG AAT ATC TTT GGT TTG GCT
      3552
      1169  Thr Ala Ser Gly Ala Leu Lys Val Ile Pro Asn Ile Phe Gly Leu Ala
      1184
45

      3553  GAT GGC GGC TCG CGC TAT GAA GGA GTA ACG GAA GCG ATT GCC ATC GGG
      3600
      1185  Asp Gly Gly Ser Arg Tyr Glu Gly Val Thr Glu Ala Ile Ala Ile Gly
50    1200

      3601  TTA ATG GCT GCC GGA CAA GCC ACC AGC GTG GTG GCC GAG CGT CTG GCA
      3648
55    1201  Leu Met Ala Ala Gly Gln Ala Thr Ser Val Val Ala Glu Arg Leu Ala
      1216

      3649  ACC ACG GAG AAT TAC CGC CGC CGC CGT GAA GAG TGG CAA ATC CAA TAC
60    3696
      1217  Thr Thr Glu Asn Tyr Arg Arg Arg Arg Glu Glu Trp Gln Ile Gln Tyr
      1232

65    3697  CAG CAG GCA CAG TCT GAG GTC GAC GCA TTA CAG AAA CAG TTG GAT GCG
      3744
      1233  Gln Gln Ala Gln Ser Glu Val Asp Ala Leu Gln Lys Gln Leu Asp Ala
      1248

70
```

SUBSTITUTE SHEET (RULE 26)

3745 CTG GCA GTG CGC GAG AAA GCA GCT CAA ACT TCC CTG CAA CAG GCG AAG
3792
1249 Leu Ala Val Arg Glu Lys Ala Ala Gln Thr Ser Leu Gln Gln Ala Lys
1264

3793 GCA CAG CAG GTA CAA ATT CGG ACC ATG CTG ACT TAC TTA ACT ACT CGT
3840
1265 Ala Gln Gln Val Gln Ile Arg Thr Met Leu Thr Tyr Leu Thr Thr Arg
1280

3841 TTC ACC CAG GCG ACT CTG TAC CAG TGG CTG AGT GGT CAA TTA TCC GCG
3888
1281 Phe Thr Gln Ala Thr Leu Tyr Gln Trp Leu Ser Gly Gln Leu Ser Ala
1296

3889 TTG TAT TAT CAA GCG TAT GAT GCC GTG GTT GCT CTC TGC CTC TCC GCC
3936
1297 Leu Tyr Tyr Gln Ala Tyr Asp Ala Val Val Ala Leu Cys Leu Ser Ala
1312

3937 CAA GCT TGC TGG CAG TAT GAA TTG GGT GAT TAC GCT ACC ACT TTT ATC
3984
1313 Gln Ala Cys Trp Gln Tyr Glu Leu Gly Asp Tyr Ala Thr Thr Phe Ile
1328

3985 CAG ACC GGT ACC TGG AAC GAC CAT TAC CGT GGT TTG CAA GTG GGG GAG
4032
1329 Gln Thr Gly Thr Trp Asn Asp His Tyr Arg Gly Leu Gln Val Gly Glu
1344

4033 ACA CTG CAA CTC AAT TTG CAT CAG ATG GAA GCG GCC TAT TTA GTT CGT
4080
1345 Thr Leu Gln Leu Asn Leu His Gln Met Glu Ala Ala Tyr Leu Val Arg
1360

4081 CAC GAA CGC CGT CTT AAT GTG ATC CGT ACT GTG TCG CTC AAA AGC CTA
4128
1361 His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu
1376

4129 TTG GGT GAT GAT GGT TTT GGT AAG TTA AAA ACC GAA GGC AAA GTC GAC
4176
1377 Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp
1392

4177 TTT CCA TTA AGC GAA AAG CTG TTT GAC AAC GAC TAT CCG GGG CAC TAT
4224
1393 Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr
1408

4225 TTG CGC CAG ATT AAA ACT GTG TCA GTG ACG TTG CCG ACG TTA GTC GGG
4272
1409 Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly
1424

4273 CCG TAT CAA AAC GTG AAG GCA ACG CTC ACT CAG ACC AGC AGC AGT ATA
4320
1425 Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile
1440

SUBSTITUTE SHEET (RULE 26)

```
4321 TTG TTA GCA GCA GAT ATC AAT GGT GTT AAA CGT CTC AAT GAT CCG ACA
4368
1441 Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr
1456


4369 GGT AAA GAG GGT GAT GCG ACG CAT ATT GTC ACC AAT CTG CGT GCC AGC
4416
1457 Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser
1472


4417 CAG CAG GTG GCG CTC TCT TCT GGC ATT AAT GAT GCC GGT AGC TTT GAG
4464
1473 Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu
1488


4465 TTG CGT TTG GAA GAT GAG CGC TAT CTA TCA TTT GAG GGG ACT GGA GCT
4512
1489 Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala
1504


4513 GTT TCC AAA TGG ACT CTT AAC TTC CCG CGT TCT GTG GAT GAG CAT ATT
4560
1505 Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile
1520


4561 GAC GAT AAG ACA TTG AAA GCG GAT GAG ATG CAG GCC GCA CTG TTG GCG
4608
1521 Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala
1536


4609 AAT ATG GAT GAT GTG CTG GTG CAG GTG CAT TAT ACC GCC TGC GAC GGC
4656
1537 Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly
1552


4657 GGC GCC AGT TTC GCA AAC CAG GTC AAG AAA ACA CTC TCT TAA CATTAACTTT 4708
1553 Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser End          1565


4709 TAACTAATCC CTCCCACTCT GTTCGCCAGA GTGGGAGAAG GTTTGTCATA TCTAAAATCA 4768


4770 ATCTTGCGAT CTTTCTCCAT TTCATTGGAA GGGAAGCTGT AAAACAAATA AGGAATATGA 4828


4829 TATG                                                             4932
```

(2) INFORMATION FOR SEQ ID NO:59

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1565 amino acids
        (B) TYPE: amino acid
        (C) TOPOLOGY: linear
   (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59 (TccB peptide)
        Features   From   To   Description

   1 Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp Ala
16

SUBSTITUTE SHEET (RULE 26)

```
        17   Leu Val Thr Gly Tyr Met Asn Phe Val Ala Pro Thr Leu Lys Gly Val
        32
  5     33   Ser Gly Gln Pro Val Thr Val Glu Asp Leu Tyr Glu Tyr Leu Leu Ile
        48

        49   Asp Pro Glu Val Ala Asp Glu Val Glu Thr Ser Arg Val Ala Gln Ala
        64
 10     65   Ile Ala Ser Ile Gln Gln Tyr Met Thr Arg Leu Val Asn Gly Ser-Glu
        80

        81   Pro Gly Arg Gln Ala Met Glu Pro Ser Thr Ala Asn Glu Trp Arg Asp
 15     96

        97   Asn Asp Asn Gln Tyr Ala Ile Trp Ala Ala Gly Ala Glu Val Arg Asn
       112
 20    113   Tyr Ala Glu Asn Tyr Ile Ser Pro Ile Thr Arg Gln Glu Lys Ser His
       128

       129   Tyr Phe Ser Glu Leu Glu Thr Thr Leu Asn Gln Asn Arg Leu Asp Pro
       144
 25    145   Asp Arg Val Gln Asp Ala Val Leu Ala Tyr Leu Asn Glu Phe Glu Ala
       160

       161   Val Ser Asn Leu Tyr Val Leu Ser Gly Tyr Ile Asn Gln Asp Lys Phe
 30    176

       177   Asp Gln Ala Ile Tyr Tyr Phe Ile Gly Arg Thr Thr Thr Lys Pro Tyr
       192
 35    193   Arg Tyr Tyr Trp Arg Gln Met Asp Leu Ser Lys Asn Arg Gln Asp Pro
       208

       209   Ala Gly Asn Pro Val Thr Pro Asn Cys Trp Asn Asp Trp Gln Glu Ile
       224
 40    225   Thr Leu Pro Leu Ser Gly Asp Thr Val Leu Glu His Thr Val Arg Pro
       240

       241   Val Phe Tyr Asn Asp Arg Leu Tyr Val Ala Trp Val Glu Arg Asp Pro
 45    256

       257   Ala Val Gln Lys Asp Ala Asp Gly Lys Asn Ile Gly Lys Thr His Ala
       272
 50    273   Tyr Asn Ile Lys Phe Gly Tyr Lys Arg Tyr Asp Asp Thr Trp Thr Ala
       288

       289   Pro Asn Thr Thr Thr Leu Met Thr Gln Gln Ala Gly Glu Ser Ser Glu
       304
 55    305   Thr Gln Arg Ser Ser Leu Leu Ile Asp Glu Ser Ser Thr Thr Leu Arg
       320

       321   Gln Val Asn Leu Leu Ala Thr Thr Asp Phe Ser Ile Asp Pro Thr Glu
 60    336

       337   Glu Thr Asp Ser Asn Pro Tyr Gly Arg Leu Met Leu Gly Val Phe Val
       352
 65    353   Arg Gln Phe Glu Gly Asp Gly Ala Asn Arg Lys Asn Lys Pro Val Val
       368

       369   Tyr Gly Tyr Leu Tyr Cys Asp Ser Ala Phe Asn Arg His Val Leu Arg
       384
 70
```

SUBSTITUTE SHEET (RULE 26)

```
        385  Pro Leu Ser Lys Asn Phe Leu Phe Ser Thr Tyr Arg Asp Glu Thr Asp
        400

   5    401  Gly Gln Asn Ser Leu Gln Phe Ala Val Tyr Asp Lys Lys Tyr Val Ile
        416

        417  Thr Lys Val Val Thr Gly Ala Thr Glu Asp Pro Glu Asn Thr Gly Trp
        432

  10    433  Val Ser Lys Val Asp Asp Leu Lys Gln Gly Thr Thr Gly Ala Tyr Val
        448

        449  Tyr Ile Asp Gln Asp Gly Leu Thr Leu His Ile Gln Thr Thr Thr Asn
        464

  15    465  Gly Asp Phe Ile Asn Arg His Thr Phe Gly Tyr Asn Asp Leu Val Tyr
        480

        481  Asp Ser Lys Ser Gly Tyr Gly Phe Thr Trp Ser Gly Asn Glu Gly Phe
  20    496

        497  Tyr Leu Asp Tyr His Asp Gly Asn Tyr Tyr Thr Phe His Asn Ala Ile
        512

  25    513  Ile Asn Tyr Tyr Pro Ser Gly Tyr Gly Gly Gly Ser Val Pro Asn Gly
        528

        529  Thr Trp Ala Leu Glu Gln Arg Ile Asn Glu Gly Trp Ala Ile Ala Pro
        544
  30
        545  Leu Leu Asp Thr Leu His Thr Val Thr Val Lys Gly Ser Tyr Ile Ala
        560

        561  Trp Glu Gly Glu Thr Pro Thr Gly Tyr Asn Leu Tyr Ile Pro Asp Gly
  35    576

        577  Thr Val Leu Leu Asp Trp Phe Asp Lys Ile Asn Phe Ala Ile Gly Leu
        592

  40    593  Asn Lys Leu Glu Ser Val Phe Thr Ser Pro Asp Trp Pro Thr Leu Thr
        608

        609  Thr Ile Lys Asn Phe Ser Lys Ile Ala Asp Asn Arg Lys Phe Tyr Gln
        624
  45
        625  Glu Ile Asn Ala Glu Thr Ala Asp Gly Arg Asn Leu Phe Lys Arg Tyr
        640

        641  Ser Thr Gln Thr Phe Gly Leu Thr Ser Gly Ala Thr Tyr Ser Thr Thr
  50    656

        657  Tyr Thr Leu Ser Glu Ala Asp Phe Ser Thr Asp Pro Asp Lys Asn Tyr
        672

  55    673  Leu Gln Val Cys Leu Asn Val Val Trp Asp His Tyr Asp Arg Pro Ser
        688

        689  Gly Lys Lys Gly Ala Tyr Ser Trp Val Ser Lys Trp Phe Asn Val Tyr
        704
  60
        705  Val Ala Leu Gln Asp Ser Lys Ala Pro Asp Ala Ile Pro Arg Leu Val
        720

        721  Ser Arg Tyr Asp Ser Lys Arg Gly Leu Val Gln Tyr Leu Asp Phe Trp
  65    736

        737  Thr Ser Ser Leu Pro Ala Lys Thr Arg Leu Asn Thr Thr Phe Val Arg
        752

  70    753  Thr Leu Ile Glu Lys Ala Asn Leu Gly Leu Asp Ser Leu Leu Asp Tyr
        768
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```
      769  Thr Leu Gln Ala Asp Pro Ser Leu Glu Ala Asp Leu Val Thr Asp Gly
      784

 5    785  Lys Ser Glu Pro Met Asp Phe Asn Gly Ser Asn Gly Leu Tyr Phe Trp
      800

      801  Glu Leu Phe Phe His Leu Pro Phe Leu Val Ala Thr Arg Phe Ala Asn
      816
10
      817  Glu Gln Gln Phe Ser Pro Ala Gln Lys Ser Leu His Tyr Ile Phe Asp
      832

      833  Pro Ala Met Lys Asn Lys Pro His Asn Ala Pro Ala Tyr Trp Asn Val
15    848

      849  Arg Pro Leu Val Glu Gly Asn Ser Asp Leu Ser Arg His Leu Asp Asp
      864

20    865  Ser Ile Asp Pro Asp Thr Gln Ala Tyr Ala His Pro Val Ile Tyr Gln
      880

      881  Lys Ala Val Phe Ile Ala Tyr Val Ser Asn Leu Ile Ala Gln Gly Asp
      896
25
      897  Met Trp Tyr Arg Gln Leu Thr Arg Asp Gly Leu Thr Gln Ala Arg Val
      912

      913  Tyr Tyr Asn Leu Ala Ala Glu Leu Leu Gly Pro Arg Pro Asp Val Ser
30    928

      929  Leu Ser Ser Ile Trp Thr Pro Gln Thr Leu Asp Thr Leu Ala Ala Gly
      944

35    945  Gln Lys Ala Val Leu Arg Asp Phe Glu His Gln Leu Ala Asn Ser Asp
      960

      961  Thr Ala Leu Pro Ala Leu Pro Gly Arg Asn Val Ser Tyr Leu Lys Leu
      976
40
      977  Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val Leu Met Leu Ser
      992

      993  His Trp Asp Thr Leu Asp Ala Arg Leu Tyr Asn Leu Arg His Asn Leu
45    1008

      1009 Thr Val Asp Gly Lys Pro Leu Ser Leu Pro Leu Tyr Ala Ala Pro Val
      1024

50    1025 Asp Pro Val Ala Leu Leu Ala Gln Arg Ala Gln Ser Gly Thr Leu Thr
      1040

      1041 Asn Gly Val Ser Gly Ala Met Leu Thr Val Pro Pro Tyr Arg Phe Ser
      1056
55
      1057 Ala Met Leu Pro Arg Ala Tyr Ser Ala Val Gly Thr Leu Thr Ser Phe
      1072

      1073 Gly Gln Asn Leu Leu Ser Leu Leu Glu Arg Ser Glu Arg Ala Cys Gln
60    1088

      1089 Glu Glu Leu Ala Gln Gln Gln Leu Leu Asp Met Ser Ser Tyr Ala Ile
      1104

65    1105 Thr Leu Gln Gln Gln Ala Leu Asp Gly Leu Ala Ala Asp Arg Leu Ala
      1120

      1121 Leu Leu Ala Ser Gln Ala Thr Ala Gln Gln Arg His Asp His Tyr Tyr
      1136
70
```

SUBSTITUTE SHEET (RULE 26)

	1137 1152	Thr Leu Tyr Gln Asn Asn Ile Ser Ser Ala Glu Gln Leu Val Met Asp
5	1153 1168	Thr Gln Thr Ser Ala Gln Ser Leu Ile Ser Ser Ser Thr Gly Val Gln
	1169 1184	Thr Ala Ser Gly Ala Leu Lys Val Ile Pro Asn Ile Phe Gly Leu Ala
10	1185 1200	Asp Gly Gly Ser Arg Tyr Glu Gly Val Thr Glu Ala Ile Ala Ile Gly
	1201 1216	Leu Met Ala Ala Gly Gln Ala Thr Ser Val Val Ala Glu Arg Leu Ala
15	1217 1232	Thr Thr Glu Asn Tyr Arg Arg Arg Arg Glu Glu Trp Gln Ile Gln Tyr
20	1233 1248	Gln Gln Ala Gln Ser Glu Val Asp Ala Leu Gln Lys Gln Leu Asp Ala
	1249 1264	Leu Ala Val Arg Glu Lys Ala Ala Gln Thr Ser Leu Gln Gln Ala Lys
25	1265 1280	Ala Gln Gln Val Gln Ile Arg Thr Met Leu Thr Tyr Leu Thr Thr Arg
30	1281 1296	Phe Thr Gln Ala Thr Leu Tyr Gln Trp Leu Ser Gly Gln Leu Ser Ala
	1297 1312	Leu Tyr Tyr Gln Ala Tyr Asp Ala Val Val Ala Leu Cys Leu Ser Ala
35	1313 1328	Gln Ala Cys Trp Gln Tyr Glu Leu Gly Asp Tyr Ala Thr Thr Phe Ile
	1329 1344	Gln Thr Gly Thr Trp Asn Asp His Tyr Arg Gly Leu Gln Val Gly Glu
40	1345 1360	Thr Leu Gln Leu Asn Leu His Gln Met Glu Ala Ala Tyr Leu Val Arg
45	1361 1376	His Glu Arg Arg Leu Asn Val Ile Arg Thr Val Ser Leu Lys Ser Leu
	1377 1392	Leu Gly Asp Asp Gly Phe Gly Lys Leu Lys Thr Glu Gly Lys Val Asp
50	1393 1408	Phe Pro Leu Ser Glu Lys Leu Phe Asp Asn Asp Tyr Pro Gly His Tyr
	1409 1424	Leu Arg Gln Ile Lys Thr Val Ser Val Thr Leu Pro Thr Leu Val Gly
55	1425 1440	Pro Tyr Gln Asn Val Lys Ala Thr Leu Thr Gln Thr Ser Ser Ser Ile
60	1441 1456	Leu Leu Ala Ala Asp Ile Asn Gly Val Lys Arg Leu Asn Asp Pro Thr
	1457 1472	Gly Lys Glu Gly Asp Ala Thr His Ile Val Thr Asn Leu Arg Ala Ser
65	1473 1488	Gln Gln Val Ala Leu Ser Ser Gly Ile Asn Asp Ala Gly Ser Phe Glu
	1489 1504	Leu Arg Leu Glu Asp Glu Arg Tyr Leu Ser Phe Glu Gly Thr Gly Ala
70	1505 1520	Val Ser Lys Trp Thr Leu Asn Phe Pro Arg Ser Val Asp Glu His Ile

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```
     1521  Asp Asp Lys Thr Leu Lys Ala Asp Glu Met Gln Ala Ala Leu Leu Ala
     1536

     1537  Asn Met Asp Asp Val Leu Val Gln Val His Tyr Thr Ala Cys Asp Gly
     1552

     1553  Gly Ala Ser Phe Ala Asn Gln Val Lys Lys Thr Leu Ser    1565
```

(2) INFORMATION FOR SEQ ID NO:60

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 3132 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60 (*tccC*)

```
     1  ATG AGT CCG TCT GAG ACT ACT CTT TAT ACT CAA ACC CCA ACA GTC AGC   48
     1  Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser   16

    49  GTG TTA GAT AAT CGC GGT CTG TCC ATT CGT GAT ATT GGT TTT CAC CGT   96
    17  Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg   32

    97  ATT GTA ATC GGG GGG GAT ACT GAC ACC CGC GTC ACC CGT CAC CAG TAT
   144
    33  Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr   48

   145  GAT GCC CGT GGA CAC CTG AAC TAC AGT ATT GAC CCA CGC TTG TAT GAT
   192
    49  Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp   64

   193  GCA AAG CAG GCT GAT AAC TCA GTA AAG CCT AAT TTT GTC TGG CAG CAT
   240
    65  Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His   80

   241  GAT CTG GCC GGT CAT GCC CTG CGG ACA GAG AGT GTC GAT GCT GGT CGT
   288
    81  Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg   96

   289  ACT GTT GCA TTG AAT GAT ATT GAA GGT CGT TCG GTA ATG ACA ATG AAT
   336
    97  Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn
   112

   337  GCG ACC GGT GTT CGT CAG ACC CGT CGC TAT GAA GGC AAC ACC TTG CCC
   384
   113  Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro
   128

   385  GGT CGC TTG TTA TCT GTG AGC GAG CAA GTT TTC AAC CAA GAG AGT GCT
   432
   129  Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala
   144

   433  AAA GTG ACA GAG CGC TTT ATC TGG GCT GGG AAT ACA ACC TCG GAG AAA
   480
```

145 Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys
160

481 GAG TAT AAC CTC TCC GGT CTG TGT ATA CGC CAC TAC GAC ACA GCG GGA
528
161 Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly
176

529 GTG ACC CGG TTG ATG AGT CAG TCA CTG GCG GGC GCC ATG CTA TCC CAA
576
177 Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln
192

577 TCT CAC CAA TTG CTG GCG GAA GGG CAG GAG GCT AAC TGG AGC GGT GAC
624
193 Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp
208

625 GAC GAA ACT GTC TGG CAG GGA ATG CTG GCA AGT GAG GTC TAT ACG ACA
672
209 Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr
224

673 CAA AGT ACC ACT AAT GCC ATC GGG GCT TTA CTG ACC CAA ACC GAT GCG
720
225 Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala
240

721 AAA GGC AAT ATT CAG CGT CTG GCT TAT GAC ATT GCC GGT CAG TTA AAA
768
241 Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys
256

769 GGG AGT TGG TTG ACG GTG AAA GGC CAG AGT GAA CAG GTG ATT GTT AAG
816
257 Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys
272

817 TCC CTG AGC TGG TCA GCC GCA GGT CAT AAA TTG CGT GAA GAG CAC GGT
864
273 Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly
288

865 AAC GGC GTG GTT ACG GAG TAC AGT TAT GAG CCG GAA ACT CAA CGT CTG
912
289 Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu
304

913 ATA GGT ATC ACC ACC CGG CGT GCC GAA GGG AGT CAA TCA GGA GCC AGA
960
305 Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg
320

961 GTA TTG CAG GAT CTA CGC TAT AAG TAT GAT CCG GTG GGG AAT GTT ATC
1008
321 Val Leu Gln Asp Leu Arg Tyr Lys Tyr Asp Pro Val Gly Asn Val Ile   336

1009 AGT ATC CAT AAT GAT GCC GAA GCT ACC CGC TTT TGG CGT AAT CAG AAA
1056

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```
    337  Ser Ile His Asn Asp Ala Glu Ala Thr Arg Phe Trp Arg Asn Gln Lys  352

    1057 GTG GAG CCG GAG AAT CGC TAT GTT TAT GAT TCT CTG TAT CAG CTT ATG
 5  1104
    353  Val Glu Pro Glu Asn Arg Tyr Val Tyr Asp Ser Leu Tyr Gln Leu Met  368

    1105 AGT GCG ACA GGG CGT GAA ATG GCT AAT ATC GGT CAG CAA AGC AAC CAA
10  1152
    369  Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Gly Gln Gln Ser Asn Gln
    384

    1153 CTT CCC TCA CCC GTT ATA CCT GTT CCT ACT GAC GAC AGC ACT TAT ACC
15  1200
    385  Leu Pro Ser Pro Val Ile Pro Val Pro Thr Asp Asp Ser Thr Tyr Thr  400

    1201 AAT TAC CTT CGT ACC TAT ACT TAT GAC CGT GGC GGT AAT TTG GTT CAA
20  1248
    401  Asn Tyr Leu Arg Thr Tyr Thr Tyr Asp Arg Gly Gly Asn Leu Val Gln  416

    1249 ATC CGA CAC AGT TCA CCC GCG ACT CAA AAT AGT TAC ACC ACA GAT ATC
25  1296
    417  Ile Arg His Ser Ser Pro Ala Thr Gln Asn Ser Tyr Thr Thr Asp Ile  432

    1297 ACC GTT TCA AGC CGC AGT AAC CGG GCG GTA TTG AGT ACA TTA ACG ACA
30  1344
    433  Thr Val Ser Ser Arg Ser Asn Arg Ala Val Leu Ser Thr Leu Thr Thr  448

    1345 GAT CCA ACC CGA GTG GAT GCG CTA TTT GAT TCC GGC GGT CAT CAG AAG
35  1392
    449  Asp Pro Thr Arg Val Asp Ala Leu Phe Asp Ser Gly Gly His Gln Lys  464

    1393 ATG TTA ATA CCG GGG CAA AAT CTG GAT TGG AAT ATT CGG GGT GAA TTG
40  1440
    465  Met Leu Ile Pro Gly Gln Asn Leu Asp Trp Asn Ile Arg Gly Glu Leu  480

    1441 CAA CGA GTC ACA CCG GTG AGC CGT GAA AAT AGC AGT GAC AGT GAA TGG
45  1488
    481  Gln Arg Val Thr Pro Val Ser Arg Glu Asn Ser Ser Asp Ser Glu Trp  496

    1489 TAT CGC TAT AGC AGT GAT GGC ATG CGG CTG CTA AAA GTG AGT GAA CAG
50  1536
    497  Tyr Arg Tyr Ser Ser Asp Gly Met Arg Leu Leu Lys Val Ser Glu Gln  512

    1537 CAG ACG GGC AAC AGT ACT CAA GTA CAA CGG GTG ACT TAT CTG CCG GGA
55  1584
    513  Gln Thr Gly Asn Ser Thr Gln Val Gln Arg Val Thr Tyr Leu Pro Gly  528

    1585 TTA GAG CTA CGG ACA ACT GGG GTT GCA GAT AAA ACA ACC GAA GAT TTG
60  1632
    529  Leu Glu Leu Arg Thr Thr Gly Val Ala Asp Lys Thr Thr Glu Asp Leu  544

    1633 CAG GTG ATT ACG GTA GGT GAA GCG GGT CGC GCA CAG GTA AGG GTA TTG
65  1680
    545  Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu  560

    1681 CAC TGG GAA AGT GGT AAG CCG ACA GAT ATT GAC AAC AAT CAG GTG CGC
70  1728
```

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```
          561  His Trp Glu Ser Gly Lys Pro Thr Asp Ile Asp Asn Asn Gln Val Arg  576

     5   1729  TAC AGC TAC GAT AAT CTG CTT GGC TCC AGC CAG CTT GAA CTG GAT AGC
         1776
          577  Tyr Ser Tyr Asp Asn Leu Leu Gly Ser Ser Gln Leu Glu Leu Asp Ser  592

    10   1777  GAA GGG CAG ATT CTC AGT CAG GAA GAG TAT TAT CCG TAT GGC GGT ACG
         1824
          593  Glu Gly Gln Ile Leu Ser Gln Glu Glu Tyr Tyr Pro Tyr Gly Gly Thr  608

    15   1825  GCG ATA TGG GCG GCG AGA AAT CAG ACA GAA GCC AGC TAC AAA TTT ATT
         1872
          609  Ala Ile Trp Ala Ala Arg Asn Gln Thr Glu Ala Ser Tyr Lys Phe Ile  624

    20   1873  CGT TAC TCC GGT AAA GAG CGG GAT GCC ACT GGA TTG TAT TAT TAC GGC
         1920
          625  Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly Leu Tyr Tyr Tyr Gly  640

    25   1921  TAC CGT TAT TAT CAA CCT TGG GTG GGT CGA TGG TTG AGT GCT GAT CCG
         1968
          641  Tyr Arg Tyr Tyr Gln Pro Trp Val Gly Arg Trp Leu Ser Ala Asp Pro  656

    30   1969  GCG GGA ACC GTG GAT GGG CTG AAT TTG TAC CGA ATG GTG AGG AAT AAC
         2016
          657  Ala Gly Thr Val Asp Gly Leu Asn Leu Tyr Arg Met Val Arg Asn Asn  672

    35   2017  CCC ATC ACA TTG ACT GAC CAT GAC GGA TTA GCA CCG TCT CCA AAT AGA
         2064
          673  Pro Ile Thr Leu Thr Asp His Asp Gly Leu Ala Pro Ser Pro Asn Arg  688

    40   2065  AAT CGA AAT ACA TTT TGG TTT GCT TCA TTT TTG TTT CGT AAA CCT GAT
         2112
          689  Asn Arg Asn Thr Phe Trp Phe Ala Ser Phe Leu Phe Arg Lys Pro Asp  704

    45   2113  GAG GGA ATG TCC GCG TCA ATG AGA CGG GGA CAA AAA ATT GGC AGA GCC
         2160
          705  Glu Gly Met Ser Ala Ser Met Arg Arg Gly Gln Lys Ile Gly Arg Ala  720

    50   2161  ATT GCC GGC GGG ATT GCG ATT GGC GGT CTT GCG GCT ACC ATT GCC GCT
         2208
          721  Ile Ala Gly Gly Ile Ala Ile Gly Gly Leu Ala Ala Thr Ile Ala Ala  736

    55   2209  ACG GCT GGC GCG GCT ATC CCC GTC ATT CTG GGG GTT GCG GCC GTA GGC
         2256
          737  Thr Ala Gly Ala Ala Ile Pro Val Ile Leu Gly Val Ala Ala Val Gly  752

    60   2257  GCG GGG ATT GGC GCG TTG ATG GGA TAT AAC GTC GGT AGC CTG CTG GAA
         2304
          753  Ala Gly Ile Gly Ala Leu Met Gly Tyr Asn Val Gly Ser Leu Leu Glu  768

    65   2305  AAA GGC GGG GCA TTA CTT GCT CGA CTC GTA CAG GGG AAA TCG ACG TTA
         2352
          769  Lys Gly Gly Ala Leu Leu Ala Arg Leu Val Gln Gly Lys Ser Thr Leu  784

    70   2353  GTA CAG TCG GCG GCT GGC GCG GCT GCC GGA GCG AGT TCA GCC GCG GCT
         2400
          785  Val Gln Ser Ala Ala Gly Ala Ala Ala Gly Ala Ser Ser Ala Ala Ala  800
```

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SUBSTITUTE SHEET (RULE 26)

```
        2401  TAT GGC GCA CGG GCA CAA GGT GTC GGT GTT GCA TCA GCC GCC GGG GCG
        2448
 5       801  Tyr Gly Ala Arg Ala Gln Gly Val Gly Val Ala Ser Ala Ala Gly Ala  816


        2449  GTA ACA GGG GCT GTG GGA TCA TGG ATA AAT AAT GCT GAT CGG GGG ATT
        2496
10       817  Val Thr Gly Ala Val Gly Ser Trp Ile Asn Asn Ala Asp Arg Gly Ile  832


        2497  GGC GGC GCT ATT GGG GCC GGG AGT GCG GTA GGC ACC ATT GAT ACT ATG
        2544
15       833  Gly Gly Ala Ile Gly Ala Gly Ser Ala Val Gly Thr Ile Asp Thr Met  848


        2545  TTA GGG ACT GCC TCT ACC CTT ACC CAT GAA GTC GGG GCA GCG GCG GGT
        2592
20       849  Leu Gly Thr Ala Ser Thr Leu Thr His Glu Val Gly Ala Ala Ala Gly  864


        2593  GGG GCG GCG GGT GGG ATG ATC ACC GGT ACG CAA GGG AGT ACT CGG GCA
        2640
25       865  Gly Ala Ala Gly Gly Met Ile Thr Gly Thr Gln Gly Ser Thr Arg Ala  880


        2641  GGT ATC CAT GCC GGT ATT GGC ACC TAT TAT GGC TCC TGG ATT GGT TTT
        2688
30       881  Gly Ile His Ala Gly Ile Gly Thr Tyr Tyr Gly Ser Trp Ile Gly Phe  896


        2689  GGT TTA GAT GTC GCT AGT AAC CCC GCC GGA CAT TTA GCG AAT TAC GCA
        2736
35       897  Gly Leu Asp Val Ala Ser Asn Pro Ala Gly His Leu Ala Asn Tyr Ala  912


        2737  GTG GGT TAT GCC GCT GGT TTG GGT GCT GAA ATG GCT GTC AAC AGA ATA
        2784
40       913  Val Gly Tyr Ala Ala Gly Leu Gly Ala Glu Met Ala Val Asn Arg Ile  928


        2785  ATG GGT GGT GGA TTT TTG AGT AGG CTC TTA GGC CGG GTT GTC AGC CCA
        2832
45       929  Met Gly Gly Gly Phe Leu Ser Arg Leu Leu Gly Arg Val Val Ser Pro  944


        2833  TAT GCC GCC GGT TTA GCC AGA CAA TTA GTA CAT TTC AGT GTC GCC AGA
        2880
50       945  Tyr Ala Ala Gly Leu Ala Arg Gln Leu Val His Phe Ser Val Ala Arg  960


        2881  CCT GTC TTT GAG CCG ATA TTT AGT GTT CTC GGC GGG CTT GTC GGT GGT
        2928
55       961  Pro Val Phe Glu Pro Ile Phe Ser Val Leu Gly Gly Leu Val Gly Gly  976


        2929  ATT GGA ACT GGC CTG CAC AGA GTG ATG GGA AGA GAG AGT TGG ATT TCC
        2976
60       977  Ile Gly Thr Gly Leu His Arg Val Met Gly Arg Glu Ser Trp Ile Ser  992


        2977  AGA GCG TTA AGT GCT GCC GGT AGT GGT ATA GAT CAT GTC GCT GGC ATG
        3024
65       993  Arg Ala Leu Ser Ala Ala Gly Ser Gly Ile Asp His Val Ala Gly Met  1008
        1008


        3025  ATT GGT AAT CAG ATC AGA GGC AGG GTC TTG ACC ACA ACC GGG ATC GCT
70      3072
```

SUBSTITUTE SHEET (RULE 26)

```
1009   Ile Gly Asn Gln Ile Arg Gly Arg Val Leu Thr Thr Thr Gly Ile Ala
1024
```

```
3073   AAT GCG ATA GAC TAT GGC ACC AGT GCT GTG GGA GCC GCA CGA CGA GTT
3120
1025   Asn Ala Ile Asp Tyr Gly Thr Ser Ala Val Gly Ala Ala Arg Arg Val
1040
```

```
3121   TTT TCT TTG TAA   3132
1041   Phe Ser Leu End   1043
```

(2) INFORMATION FOR SEQ ID NO:61

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1043 amino acids
        (B) TYPE: amino acid
        (C) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61 (TccC peptide)

```
  1   Met Ser Pro Ser Glu Thr Thr Leu Tyr Thr Gln Thr Pro Thr Val Ser    16

 17   Val Leu Asp Asn Arg Gly Leu Ser Ile Arg Asp Ile Gly Phe His Arg    32

 33   Ile Val Ile Gly Gly Asp Thr Asp Thr Arg Val Thr Arg His Gln Tyr    48

 49   Asp Ala Arg Gly His Leu Asn Tyr Ser Ile Asp Pro Arg Leu Tyr Asp    64

 65   Ala Lys Gln Ala Asp Asn Ser Val Lys Pro Asn Phe Val Trp Gln His    80

 81   Asp Leu Ala Gly His Ala Leu Arg Thr Glu Ser Val Asp Ala Gly Arg    96

 97   Thr Val Ala Leu Asn Asp Ile Glu Gly Arg Ser Val Met Thr Met Asn   112

113   Ala Thr Gly Val Arg Gln Thr Arg Arg Tyr Glu Gly Asn Thr Leu Pro   128

129   Gly Arg Leu Leu Ser Val Ser Glu Gln Val Phe Asn Gln Glu Ser Ala   144

145   Lys Val Thr Glu Arg Phe Ile Trp Ala Gly Asn Thr Thr Ser Glu Lys   160

161   Glu Tyr Asn Leu Ser Gly Leu Cys Ile Arg His Tyr Asp Thr Ala Gly   176

177   Val Thr Arg Leu Met Ser Gln Ser Leu Ala Gly Ala Met Leu Ser Gln   192

193   Ser His Gln Leu Leu Ala Glu Gly Gln Glu Ala Asn Trp Ser Gly Asp   208

209   Asp Glu Thr Val Trp Gln Gly Met Leu Ala Ser Glu Val Tyr Thr Thr   224

225   Gln Ser Thr Thr Asn Ala Ile Gly Ala Leu Leu Thr Gln Thr Asp Ala   240

241   Lys Gly Asn Ile Gln Arg Leu Ala Tyr Asp Ile Ala Gly Gln Leu Lys   256

257   Gly Ser Trp Leu Thr Val Lys Gly Gln Ser Glu Gln Val Ile Val Lys   272

273   Ser Leu Ser Trp Ser Ala Ala Gly His Lys Leu Arg Glu Glu His Gly   288

289   Asn Gly Val Val Thr Glu Tyr Ser Tyr Glu Pro Glu Thr Gln Arg Leu   304

305   Ile Gly Ile Thr Thr Arg Arg Ala Glu Gly Ser Gln Ser Gly Ala Arg   320

321   Val Leu Gln Asp Leu Arg Tyr Lys Tyr Asp Pro Val Gly Asn Val Ile   336

337   Ser Ile His Asn Asp Ala Glu Ala Thr Arg Phe Trp Arg Asn Gln Lys   352
```

SUBSTITUTE SHEET (RULE 26)

```
      353  Val Glu Pro Glu Asn Arg Tyr Val Tyr Asp Ser Leu Tyr Gln Leu Met  368

      369  Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Gly Gln Gln Ser Asn Gln  384

 5    385  Leu Pro Ser Pro Val Ile Pro Val Pro Thr Asp Asp Ser Thr Tyr Thr  400

      401  Asn Tyr Leu Arg Thr Tyr Thr Tyr Asp Arg Gly Gly Asn Leu Val Gln  416

 10   417  Ile Arg His Ser Ser Pro Ala Thr Gln Asn Ser Tyr Thr Thr Asp Ile  432

      433  Thr Val Ser Ser Arg Ser Asn Arg Ala Val Leu Ser Thr Leu Thr Thr  448

      449  Asp Pro Thr Arg Val Asp Ala Leu Phe Asp Ser Gly Gly His Gln Lys  464

 15   465  Met Leu Ile Pro Gly Gln Asn Leu Asp Trp Asn Ile Arg Gly Glu Leu  480

      481  Gln Arg Val Thr Pro Val Ser Arg Glu Asn Ser Ser Asp Ser Glu Trp  496

 20   497  Tyr Arg Tyr Ser Ser Asp Gly Met Arg Leu Leu Lys Val Ser Glu Gln  512

      513  Gln Thr Gly Asn Ser Thr Gln Val Gln Arg Val Thr Tyr Leu Pro Gly  528

      529  Leu Glu Leu Arg Thr Thr Gly Val Ala Asp Lys Thr Thr Glu Asp Leu  544

 25   545  Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu  560

      561  His Trp Glu Ser Gly Lys Pro Thr Asp Ile Asp Asn Asn Gln Val Arg  576

      577  Tyr Ser Tyr Asp Asn Leu Leu Gly Ser Ser Gln Leu Glu Leu Asp Ser  592

 30   593  Glu Gly Gln Ile Leu Ser Gln Glu Glu Tyr Tyr Pro Tyr Gly Gly Thr  608

      609  Ala Ile Trp Ala Ala Arg Asn Gln Thr Glu Ala Ser Tyr Lys Phe Ile  624

 35   625  Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly Leu Tyr Tyr Tyr Gly  640

      641  Tyr Arg Tyr Tyr Gln Pro Trp Val Gly Arg Trp Leu Ser Ala Asp Pro  656

      657  Ala Gly Thr Val Asp Gly Leu Asn Leu Tyr Arg Met Val Arg Asn Asn  672

 40   673  Pro Ile Thr Leu Thr Asp His Asp Gly Leu Ala Pro Ser Pro Asn Arg  688

      689  Asn Arg Asn Thr Phe Trp Phe Ala Ser Phe Leu Phe Arg Lys Pro Asp  704

 45   705  Glu Gly Met Ser Ala Ser Met Arg Arg Gly Gln Lys Ile Gly Arg Ala  720

      721  Ile Ala Gly Gly Ile Ala Ile Gly Gly Leu Ala Ala Thr Ile Ala Ala  736

      737  Thr Ala Gly Ala Ala Ile Pro Val Ile Leu Gly Val Ala Ala Val Gly  752

 50   753  Ala Gly Ile Gly Ala Leu Met Gly Tyr Asn Val Gly Ser Leu Leu Glu  768

      769  Lys Gly Gly Ala Leu Leu Ala Arg Leu Val Gln Gly Lys Ser Thr Leu  784

 55   785  Val Gln Ser Ala Ala Gly Ala Ala Ala Gly Ala Ser Ser Ala Ala Ala  800

      801  Tyr Gly Ala Arg Ala Gln Gly Val Gly Val Ala Ser Ala Ala Gly Ala  816

      817  Val Thr Gly Ala Val Gly Ser Trp Ile Asn Asn Ala Asp Arg Gly Ile  832

 60   833  Gly Gly Ala Ile Gly Ala Gly Ser Ala Val Gly Thr Ile Asp Thr Met  848

      849  Leu Gly Thr Ala Ser Thr Leu Thr His Glu Val Gly Ala Ala Ala Gly  864

 65   865  Gly Ala Ala Gly Gly Met Ile Thr Gly Thr Gln Gly Ser Thr Arg Ala  880

      881  Gly Ile His Ala Gly Ile Gly Thr Tyr Tyr Gly Ser Trp Ile Gly Phe  896

      897  Gly Leu Asp Val Ala Ser Asn Pro Ala Gly His Leu Ala Asn Tyr Ala  912

 70   913  Val Gly Tyr Ala Ala Gly Leu Gly Ala Glu Met Ala Val Asn Arg Ile  928
```

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SUBSTITUTE SHEET (RULE 26)

```
929   Met Gly Gly Gly Phe Leu Ser Arg Leu Leu Gly Arg Val Val Ser Pro   944

945   Tyr Ala Ala Gly Leu Ala Arg Gln Leu Val His Phe Ser Val Ala Arg   960

961   Pro Val Phe Glu Pro Ile Phe Ser Val Leu Gly Gly Leu Val Gly Gly   976

977   Ile Gly Thr Gly Leu His Arg Val Met Gly Arg Glu Ser Trp Ile Ser   992

993   Arg Ala Leu Ser Ala Ala Gly Ser Gly Ile Asp His Val Ala Gly Met  1008

1009  Ile Gly Asn Gln Ile Arg Gly Arg Val Leu Thr Thr Thr Gly Ile Ala  1024

1025  Asn Ala Ile Asp Tyr Gly Thr Ser Ala Val Gly Ala Ala Arg Arg Val  1040

1041  Phe Ser Leu   1043
```

(2) INFORMATION FOR SEQ ID NO:62: TcaA$_{iv}$

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 5 amino acids
         (B) TYPE: amino acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62: TcaA$_{iv}$

```
Asn Ile Gly Gly Asp
1               5
```

(2) INFORMATION FOR SEQ ID NO:63: TcaA$_{ii}$-syn

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 20 amino acids
         (B) TYPE: amino acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63: TcaA$_{ii}$-syn

```
Cys Leu Arg Gly Asn Ser Pro Thr Asn Pro Asp Lys Asp Gly Ile
1               5                   10              15
Phe Ala Gln Val Ala
                20
```

(2) INFORMATION FOR SEQ ID NO:64: TcaA$_{iii}$-syn

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 20 amino acids
         (B) TYPE: amino acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: Internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64: TcaA$_{iii}$-syn

```
Cys Tyr Thr Pro Asp Gln Thr Pro Ser Phe Tyr Glu Thr Ala Phe
```

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SUBSTITUTE SHEET (RULE 26)

```
        1                 5              . 10                      15
        Arg Ser Ala Asp Gly
                         20
```

(2) INFORMATION FOR SEQ ID NO:65: TcaB$_i$-syn

   (i) SEQUENCE CHARACTERISTICS:
      (A)   LENGTH: 19 amino acids
      (B)   TYPE: amino acid
      (C) STRANDEDNESS: single
      (D)   TOPOLOGY: linear
 (ii) MOLECULAR TYPE: protein
  (v) FRAGMENT TYPE: Internal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65: TcaB$_i$-syn

```
        His Gly Gln Ser Tyr Asn Asp Asn Asn Tyr Cys Asn Phe Thr Leu
        1               5                   10                  15
        Ser Ile Asn Thr
                    19
```

(2) INFORMATION FOR SEQ ID NO:66: TcaB$_{ii}$-syn

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 20 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: protein
  (v) FRAGMENT TYPE: internal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66: TcaB$_{ii}$-syn

```
        Cys Val Asp Pro Lys Thr Leu Gln Arg Gln Gln Ala Gly Gly Asp
        1               5                   10                  15
        Gly Thr Gly Ser Ser
                        20
```

(2) INFORMATION FOR SEQ ID NO:67: TcaC-syn

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 20 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: protein
  (v) FRAGMENT TYPE: internal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67: TcaC-syn

```
        Cys Tyr Lys Ala Pro Gln Arg Gln Glu Asp Gly Asp Ser Asn Ala
        1               5                   10                  15
        Val Thr Tyr Asp Lys
                        20
```

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(2) INFORMATION FOR SEQ ID NO:68: TcbA$_{ii}$-syn

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULAR TYPE: protein
    (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68: TcbA$_{ii}$-syn

```
Cys Tyr Asn Glu Asn Pro Ser Ser Glu Asp Lys Lys Trp Tyr Phe
1               5               10              15
Ser Ser Lys Asp Asp
                20
```

(2) INFORMATION FOR SEQ ID NO:69: TcbA$_{iii}$-syn

    (i) SEQUENCE CHARACTERISTICS:
        (A)   LENGTH: 20 amino acids
        (B)   TYPE: amino acid
        (C)   STRANDEDNESS: single
        (D)   TOPOLOGY: linear
   (ii) MOLECULAR TYPE: protein
    (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69: TcbA$_{iii}$-syn

```
Cys Phe Asp Ser Tyr Ser Gln Leu Tyr Glu Glu Asn Ile Asn Ala
1               5               10              15
Gly Glu Gln Arg Ala
                20
```

(2) INFORMATION FOR SEQ ID NO:70: TcdA$_{ii}$-syn

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULAR TYPE: protein
    (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70: TcdA$_{ii}$-syn

```
Cys Asn Pro Asn Asn Ser Ser Asn Lys Leu Met Phe Tyr Pro Val
1               5               10              15
Tyr Gln Tyr Ser Gly Asn Thr
                20
```

(2) INFORMATION FOR SEQ ID NO:71: TcdA$_{iii}$-syn

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULAR TYPE: protein
    (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71: TcdA$_{iii}$-syn

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```
        Val Ser Gln Gly Ser Gly Ser Ala Gly Ser Gly Asn Asn Asn Leu
        1               5                   10                  15
        Ala Phe Gly Ala Gly
                        20
```

    (2)   INFORMATION FOR SEQ ID NO:72:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 12 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72: 160 kDa - Hb

```
        Met Gln Asp Ser Pro Glu Val Ala Ile Thr Thr Leu
        1               5                   10
```

    (2) INFORMATION FOR SEQ ID NO:73:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 8 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73: 170 kDa - WIR

```
        Met Gln Arg Ser Ser Glu Val Ser
        1               5
```

    (2) INFORMATION FOR SEQ ID NO:74:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 12 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74: 180 kDa - H9

```
        Met Gln Asp Ile Pro Glu Val Gln Leu Asn
        1               5                   10
```

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75: 170 kDa - Hm(2)
    INFORMATION FOR SEQ ID NO:75:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 12 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

```
        Met Gln Asp Ser Pro Glu Val Ser Val Thr Gln Asn
```

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                1   5   . 10

(2) INFORMATION FOR SEQ ID NO:76:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76: 74 kDa - H9

        Ser Glu Ser Leu Phe Thr Gln Ser Leu Lys Glu Ala Arg Arg Asp
        1   5   10   15

(2) INFORMATION FOR SEQ ID NO:77:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 14 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77: 71 kDa - Hb

        Met Asn Leu Ile Glu Ala Lys Leu Gln Glu Asn Arg Asp Ala
        1   5   10

(2) INFORMATION FOR SEQ ID NO:78:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78: 170 kDa - H9

        Met Leu Ser Thr Met Glu Lys Gln Leu Asn Glu Ser Gln Arg Asp
        1   5   10   15

(2) INFORMATION FOR SEQ ID NO:79:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
       (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79: 109 kDa - Hm

        Met Leu Asp Ile Met Glu Lys Gln Leu Asn Glu Ser Glu Arg Asp
        1   5   10   15

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(2) INFORMATION FOR SEQ ID NO:80:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 8 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80: 170 kDa - WX-1

        Met Gln Asp Ser Arg Glu Val Ser
        1   5

        (2) INFORMATION FOR SEQ ID NO:81:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 12 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81: 69 kDa - H9

        Leu Arg Ser Ala Xxx Ser Ala Leu Thr Thr Leu Leu
        1   5   10

(2) INFORMATION FOR SEQ ID NO:82:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 15 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82: 64 kDa - HP88

        Leu Lys Leu Ala Asp Asn Gly Tyr Phe Asn Glu Pro Leu Asn Val
        1   5   10   15

(2) INFORMATION FOR SEQ ID NO:83:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 15 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
        (ii) MOLECULAR TYPE: protein
        (v) FRAGMENT TYPE: N-terminal

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83: 70 kDa - NC-1

        Leu Lys Leu Ala Asp Asn Ser Tyr Phe Asn Glu Pro Leu Asn
        1   5   10   15

(2) INFORMATION FOR SEQ ID NO:84:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84: 60 kDa - WIR

```
Ser Lys Asp Glu Ser Lys Ala Asp Ser Gln Leu Val Tyr His Thr
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:85:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 14 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85: 58 kDa - NC-1

```
Met Lys Lys Arg Gly Leu Thr Thr Asn Ala Gly Ala Pro Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:86:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86: 60 kDa - WX-12

```
Met Leu Asn Pro Ile Val Arg Lys Phe Glu Tyr Gly Glu His Thr
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:87:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
    (ii) MOLECULAR TYPE: protein
     (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87: 60 kDa - Hm

```
Ala Glu Ile Tyr Asn Lys Asp Gly Asn Lys Leu Asp Leu Tyr Gly
1               5                   10                  15
```

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(2) INFORMATION FOR SEQ ID NO:88:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
   (ii) MOLECULAR TYPE: protein
    (v) FRAGMENT TYPE: N-terminal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88: 140 kDa - Hm

Asn Leu Ile Glu Ala Thr Leu Glu Gln Asn Leu Arg Asp Ala
1   5   10   15

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We claim:

1.   A composition, comprising an effective amount of a
Photorhabdus protein toxin that has functional activity
5   against an insect.

2.   The composition of Claim 1, wherein the *Photorhabdus*
toxin is produced by a purified culture of *Photorhabdus*, a
transgenic plant, baculovirus, or heterologous microbial host.
10

3.   The composition of Claim 2, wherein the *Photorhabdus*
toxin produced·by a purified culture of *Photorhabdus—*
luminescens.

15   4.   The composition of Claim 2, wherein the toxin is
produced from a purified culture of *Photorhabdus luminescens*
strain designated ATCC 55397.

5.   The composition of Claim 2, wherein the toxin is
20   produced by a purified culture of *Photorhabdus luminescens*
strain designated W-14.

6.   The composition of Claim 1, wherein the toxin is
produced by a purified culture of *Photorhabdus* strain
25   designated WX-1, WX-2, WX-3, WX-4, WX-5, WX6, WX-7, WX-8, WX-
9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1,
W30, WIR, B2, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC#
43951, ATCC# 43952, DEP1, DEP2, DEP3, P. zealandrica, P.
hepialus, HB-Arg, HB Oswego, HB Oswego, HB Lewiston, K-122,
30   HMGD, Indicus, GD, PWH-5, Megidis, HF-85, A. Cows, MP1, MP2,
   -    MP3, MP4, MP5, GL98, GL101, GL138, GL55, GL217, or GL257.

7.   The composition of Claim 2, wherein the toxin is
produced from a purified culture of *Photorhabdus luminescens*
35   strain designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7,
WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm,
HP88, NC-1, W30, WIR, B2, ATCC# 43948, ATCC# 43949, ATCC#
43950, ATCC# 43951, ATCC# 43952, DEP1, DEP2, DEP3, P.
zealandrica, P. hepialus, HB-Arg, HB Oswego, HB Oswego, HB
40   Lewiston, K-122, HMGD, Indicus, GD, PWH-5, Megidis, HF-85, A.
Cows, MP1, MP2, MP3, MP4, MP5, GL98, GL101, GL138, GL55,
GL217, or GL257.

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8.   The composition of Claim 1, wherein the toxin is
represented by amino acid sequence is SEQ ID NO:12.

9.   The composition of Claim 6, wherein the composition
is a mixture of one or more toxins produced from purified
cultures of *Photorhabdus*.

10.   The composition of Claim 1 or 6, wherein the insect
is of the order *Lepidoptera, Coleoptera, Hymenoptera, Diptera,
Dictyoptera, Acarina* or *Homoptera*.

11.   The composition of Claim 1 or 6, wherein the insect
species is from order *Coleoptera* and is Southern Corn
Rootworm, Western Corn Rootworm, Colorado Potato Beetle,
Mealworm, Boll Weevil or Turf Grub.

12.   The composition of Claim 1 or 6, wherein the insect
species is from order *Lepidoptera* and is Beet Armyworm, Black
Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European
Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

13.   The composition of Claim 1 or 6, wherein the toxin
is formulated as a sprayable insecticide.

14.   The composition of Claim 1 or Claim 6, wherein the
toxin is formulated as a bait matrix and delivered in an above
ground or below ground bait station.

15.   A method of controlling an insect, comprising orally
delivering to an insect an effective amount of a protein toxin
that has functional activity against an insect, wherein the
protein is produced by a purified bacterial culture of the
genus *Photorhabdus*.

16.   The method of Claim 15, wherein the bacterium is a
purified culture of *Photorhabdus luminescens*.

17.   The method of Claim 15, wherein the toxin is
produced from a purified culture of *Photorhabdus luminescens*
strain designated ATCC 55397.

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18.   The method of Claim 16, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strain designated W-14.

5   19.   The method of Claim 15, wherein the toxin is produced from a purified culture of *Photorhabdus* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm, HP88, NC-1, W30, WIR, B2, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC#

10   43951, ATCC# 43952, DEP1, DEP2, DEP3, P. zealandrica, P. hepialus, HB-Arg, HB Oswego, HB Oswego, HB Lewiston, K-122, HMGD, Indicus, GD, PWH-5, Megidis, HF-85, A. Cows, MP1, MP2, MP3, MP4, MP5, GL98, GL101, GL138, GL155, GL217, or GL257.

15

20.   The method of Claim 15, wherein the toxin is produced from a purified culture of *Photorhabdus luminescens* strains designated WX-1, WX-2, WX-3, WX-4, WX-5, WX-6, WX-7, WX-8, WX-9, WX-10, WX-11, WX-12, WX-14, WX-15, H9, Hb, Hm,

20   HP88, NC-1, W30, WIR, B2, ATCC# 43948, ATCC# 43949, ATCC# 43950, ATCC# 43951, ATCC# 43952, DEP1, DEP2, DEP3, P. zealandrica, P. hepialus, HB-Arg, HB Oswego, HB Oswego, HB Lewiston, K-122, HMGD, Indicus, GD, PWH-5, Megidis, HF-85, A. Cows, MP1, MP2, MP3, MP4, MP5, GL98, GL101, GL138, GL155,

25   GL217, or GL257.

21.   The method of Claim 19, wherein a mixture of one or more toxins is produced from a purified culture of *Photorhabdus* and said toxins are orally delivered to an

30   insect.

22.   The method of Claim 15, wherein the toxin is produced by a prokaryotic host transformed with a gene encoding the toxin.

35

23.   The method of Claim 15, wherein the toxin is produced by a eukaryotic host transformed with a gene encoding the toxin.

40   24.   The method of Claim 23, wherein the eukaryotic host is baculovirus.

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25.   The method of Claim 15 or 19, wherein the insect is
of the order *Lepidoptera, Coleoptera, Hymenoptera, Diptera,
Dictyoptera, Acarina* or *Homoptera.*

5   26.   The method of Claim 15 or 19, wherein the insect
species is from order *Coleoptera* and is Southern Corn
Rootworm, Western Corn Rootworm, Colorado Potato Beetle,
Mealworm, Boll Weevil or Turf Grub.

10   27.   The method of Claim 15 or 19, wherein the insect
species is from order *Lepidoptera* and is Beet Armyworm, Black
Cutworm, Cabbage Looper, Codling Moth, Corn Earworm, European
Corn Borer, Tobacco Hornworm, or Tobacco Budworm.

15   28.   The method of Claim 15 or 19, wherein the toxin is
formulated as a sprayable insecticide.

29.   The method of Claim 15 or Claim 19, wherein the
toxin is formulated as a bait matrix and delivered in an above
20   ground or below ground bait station.

30.   A method of isolating a gene coding for a protein
subunit, comprising the steps of: constructing at least one
RNA or DNA oligonucleotide molecule that corresponds to at
25   least a part of a DNA coding region of an amino acid sequence
selected from a group consisting of SEQ ID NO:1, SEQ ID NO:2,
SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID
NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO: 13,
SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID
30   NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22,
SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:36, SEQ ID NO:37, SEQ ID
NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42,
SEQ ID NO:43, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:73, SEQ ID
NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78,
35   SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID
NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87,
and SEQ ID NO:88, wherein the nucleotide molecule is used to
isolate genetic material from *Photorhabdus* or *Photorhabdus
luminescens.*
40

31.   A method for expressing a protein produced by a
purified bacterial culture of the genus *Photorhabdus* in a
prokaryotic or eukaryotic host in an effective amount so that

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the protein has functional activity against an insect, wherein
the method comprises: constructing a chimeric DNA construct
having 5' to 3' a promoter, a DNA sequence encoding a protein,
a transcription terminator, and then transferring the chimeric
5   DNA construct into the host.

32.   The method of Claim 31, wherein the protein has
functional activity against insects selected from a group
consisting of *Coleoptera*, *Lepidoptera*, *Diptera*, *Homoptera*,
10   *Hymenoptera*, *Dictyoptera*, and *Acarina*.

33.   The method of Claim 31, wherein the protein encoded
by the DNA sequence has an N-terminal amino acid sequence
selected from the group consisting of SEQ ID NO:1, SEQ ID
15   NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ
ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:
13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17,
SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID
NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:36, SEQ ID NO:37,
20   SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID
NO:42, SEQ ID NO:43, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:73,
SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID
NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82,
SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID
25   NO:87, and SEQ ID NO:88.

34.   The method of Claim 31, wherein the protein encoded
by the DNA sequence includes the amino acid sequence selected
from the group consisting of SEQ ID NO:12, SEQ ID NO:26, SEQ
30   ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID
NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53,
SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

35.   A chimeric DNA construct, adapted for expression in
35   a prokaryotic or eukaryotic host comprising, 5' to 3' a
transcriptional promoter active in the host; a DNA sequence
encoding a *Photorhabdus* protein that has functional activity
against an insect; and a transcriptional terminator.

40   36.   A chimeric DNA construct of Claim 35, wherein the
protein encoded by the DNA sequence has an N-terminal amino
acid sequence selected from the group consisting of SEQ ID
NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ

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ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10,
SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ
ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID
NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:36,
5   SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID
NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:62, SEQ ID NO:72,
SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID
NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,
SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID
10   NO:86, SEQ ID NO:87, and SEQ ID NO:88.

     37. The chimeric DNA construct of Claim 35, wherein the
protein encoded by the DNA sequence has an amino acid sequence
selected from the group consisting of SEQ ID NO:12, SEQ ID
15   NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34,
SEQ ID NO:35, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID
NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID
NO:61.

20   38. The chimeric DNA construct of Claim 35, wherein the
DNA sequence encoding the *Photorhabdus luminescens* protein is
selected from the group comprising SEQ ID NO:11, SEQ ID NO:25,
SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID
NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54,
25   SEQ ID NO:56, SEQ ID NO: 58, and SEQ ID NO:60.

     39. The chimeric DNA construct of Claim 35, wherein the
host is baculovirus or a plant cell.

30   40. An isolated and substantially purified preparation
comprising, a DNA molecule capable of encoding an effective
amount of a protein that is produced by a bacterium of the
genus *Photorhabdus* and that has functional activity against an
insect.
35

     41. The preparation of Claim 40, wherein the bacterium
is *Photorhabdus luminescens*.

     42. A purified preparation comprising, a protein
40   produced by *Photorhabdus* or *Photorhabdus luminescens* having an
N-terminal amino acid sequence selected from the group
consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ

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ID NO:9, SEQ ID NO:10, SEQ ID NO: 13,. SEQ ID NO:14, SEQ ID
NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19,
SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID
NO:24, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39,
5   SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID
NO:62, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75,
SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID
NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84,
SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88.
10

43. A purified protein preparation comprising, a protein that
has an N-terminal amino acid sequence selected from the group
consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ
15 ID NO:9, and SEQ ID NO:10,SEQ ID NO: 13, SEQ ID NO:14, SEQ ID
NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19,
SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID
NO:24, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41,
SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:62, SEQ ID NO:72, SEQ ID
20 NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,
SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID
NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86,
·SEQ ID NO:87, and SEQ ID NO:88.

25

44. A purified protein preparation comprising, a protein
selected from the group of SEQ ID NO:12, SEQ ID NO:26, SEQ ID
NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:35,
SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID
30 NO:55, SEQ ID NO:57, SEQ ID NO:59, and SEQ ID NO:61.

45. A purified DNA preparation comprising, a DNA
sequence selected from the group consisting of SEQ ID NO:11,
SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID
35 NO:33, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52,
SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58 and SEQ ID NO:60,
wherein the DNA sequence is isolated from its native host.

46. A purified protein preparation comprising, a
40 Photorhabdus luminescens protein with at least one subunit
having an approximate molecular weight between 18 kDa to about
230 kDa; between about 160 kDa to about 230 kDa; 100 kDa to

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160 kDa; about 80 kDa to about 100 kDa; or about 50 kDa to about 80 kDa.

47. A purified protein preparation comprising, a *Photorhabdus luminescens* protein with at least one subunit having an approximate molecular weight of about 280 kDa.

48. A substantially pure microorganism culture comprising, ATCC 55397.

49. The culture of Claim 48, wherein the culture is a derivative of ATCC 55397 that produces a protein toxin that has functional activity against an insect.

50. A transgenic plant comprising in its genome, a chimeric artificial gene construction imbuing the plant with an ability to express an effective amount of a *Photorhabdus* protein that has functional activity against an insect.

51. The transgenic plant of Claim 50, wherein the plant is transformed using acceleration of genetic material coated onto microparticles directly into cells, *Agrobacteria*, whiskers, or electroporation techniques

52. The transgenic plant of Claim 50, wherein the selectable marker is selected from the group consisting of kanamycin, neomycin, glyphosate, hygromycin, methotrexate, phosphinothricin (bialophos), chlorosulfuron, bromoxynil, dalapon and the like.

53. The transgenic plant of Claim 50, wherein the promoter is selected from the group consisting of octopine synthase, nopaline synthase, mannopine synthase, 35S, 19S, 35T, ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu), beta-conglycinin, phaseolin, alcohol dehydrogenase (ADH), heat-shock, ubiquitin, zein, oleosin, napin, or acyl carier protein (ACP).

54. The transgenic plant of Claim 50, wherein embryogenic tissue, callus tissue type I or II, hypocotyl, meristem, or plant tissue during dedifferentiation is used in preparing the transgenic plant.

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55. The transgenic plant of Claim 50, wherein the
chimeric gene is a DNA sequence which encodes a *Photorhabdus*
protein that has functional activity against an insect and at
5   least one codon of the gene has been modified so that the
codon is a plant preferred codon.

56. A method of controlling an insect comprising orally
delivering to an insect an effective amount of a protein
10   toxin, wherein the protein is produced by a transgenic plant,
which said insect feeds.

57. A composition of matter, comprising a purified DNA
sequence from a purified bacterial culture from the genus
15   *Photorhabdus*.

58. A substantially pure microorganism culture
comprising,
20   H9.

59. A substantially pure microorganism culture
comprising,
Hb.
25

60. A substantially pure microorganism culture
comprising,
Hm.

30   61. A substantially pure microorganism culture
comprising,
HP88.

62. A substantially pure microorganism culture
35   comprising,
NC-1.

63. A substantially pure microorganism culture
comprising,
40   W30.

64. A substantially pure microorganism culture
comprising,

WIR.

     65.   A substantially pure microorganism culture comprising,

5   B2.

     66.   A substantially pure microorganism culture comprising, P. zealandrica.

10   67.   A substantially pure microorganism culture comprising, P. hepialus.

     68.   A substantially pure microorganism culture comprising, HB-Arg.

15

     69.   A substantially pure microorganism culture comprising, HB Oswego.

     70.   A substantially pure microorganism culture

20   comprising, HB Lewiston.

     71.   A substantially pure microorganism culture comprising, K-122.

25   72.   A substantially pure microorganism culture comprising, HMGD.

     73.   A substantially pure microorganism culture comprising, Indicus.

30

     74.   A substantially pure microorganism culture comprising, GD.

     75.   A substantially pure microorganism culture

35   comprising, PWH-5.

     76.   A substantially pure microorganism culture comprising, Megidis.

40   77.   A substantially pure microorganism culture comprising, HF-85.

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78.   A substantially pure microorganism culture
comprising, A. Cows.

79.   A substantially pure microorganism culture
5   comprising, MP1.

80.   A substantially pure microorganism culture
comprising, MP2.

10   81.   A substantially pure microorganism culture
comprising, MP3.

82.   A substantially pure microorganism culture
comprising, MP4.
15

83.   A substantially pure microorganism culture
comprising, MP5.

84.   A substantially pure microorganism culture
20   comprising, GL98.

85.   A substantially pure microorganism culture
comprising, GL155.

25   86.   A substantially pure microorganism culture
comprising, GL101.

87.   A substantially pure microorganism culture
comprising, GL138.
30

88.   A substantially pure microorganism culture
comprising, GL217.

89.   A substantially pure microorganism culture
35   comprising, GL257.

90.   A method of making an antibody against a protein
fragment that is part of a protein having functional activity,
where the protein is produced by bacteria of the
40   Enterobacteracaea family, wherein the method comprises:

a)   isolating a fragment of the protein, where the
protein fragment is at least six amino acids;

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b)   immunizing a mammalian species with the protein
fragment; and

5   c)   harvesting serum containing antibody or antibody from
the spleen of the mammalian species, where the antibody
harvested is antibody to the protein fragment having
functional activity.

10   91.   The method of Claim 1, wherein the protein fragment
is selected from the group consisting of SEQ ID NO:63, SEQ ID
NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68,
SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71.

15   92.   The method of Claim 90, wherein the bacteria is from
the genus *Photorhabdus*.

93.   The method of Claim 90, wherein the bacteria is from
the genus *Photorhabdus luminescens*.
20

94.   A method of selecting a DNA fragment which encodes a
portion of a protein that has functional activity, where the
protein is produced from a bacteria of the *Enterobacteracaea*
family, wherein the method comprises:
25

a)   isolating a fragment of the DNA sequence having at
least 30 nucleotides;

b)   tagging the DNA fragment with a radioactive or
30 chemical agent;

c)   hybridizing the DNA fragment to a DNA library, where
the DNA library is an *Enterobacteracaea* cDNA or
Enterobacteracaea genomic library; and.
35

d)   selecting the fragment that is hybridized to the DNA
in the library that encodes for the protein that has
functional activity.

40   95.   The method of Claim 94, wherein the bacteria is from
the genus *Photorhabdus*.

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96. The method of Claim 95, wherein the bacteria is from the genus *Photorhabdus luminescens*.

97. A method of selecting a DNA fragment which encodes a portion of a protein that has functional activity, where the protein is produced from a bacteria of the *Enterobacteracaea* family, wherein the method comprises:

a) isolating at least two primers, where a primer is a fragment of DNA having at least twelve nucleotides;

b) using the primers from step a), amplifying a DNA fragment from *Enterobacteracaea* by using primers with polymerase chain reaction technology and purifying the DNA fragment;

c) tagging the purified DNA fragment with a radioactive or chemical agent;

d) hybridizing the purified DNA fragment to a DNA library, where the DNA library is an *Enterobacteracaea* cDNA or *Enterobacteracaea* genomic library;.and

e) selecting a DNA fragment that is equal or larger in size to the purified DNA fragment from the library, where the selected DNA fragment or portion thereof encodes for a protein that has functional activity.

98. The method of Claim 97, wherein the bacteria is from the genus *Photorhabdus*.

99. The method of Claim 98, wherein the bacteria is from the genus *Photorhabdus luminescens*.

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```
1   ATG CAG GAT TGT CCG GAA GTA TCG ATT ACA ACG CTG TCA CTT CCC AAA GGT GGC GGT
    TAC GTC CTA ACA GGC CTT CAT AGC TAA TGT TGC GAC AGT GAA GGG TTT CCA CCG CCA
1►  Met Gln Asp Cys Pro Glu Val Ser Ile Thr Thr Leu Ser Leu Pro Lys Gly Gly Gly
               P2Psh

58  GCT ATC AAT GGC ATG GCA GAA GCA CTG AAT GCT GCC GGC GGA ATG GCC TCC
    CGA TAG TTA CCG TAC CGT CTT CGT GAC TTA CGA CGG CCG CCT TAC CGG AGG
20► Ala Ile Asn Gly Met Gly Glu Ala Leu Asn Ala Ala Gly Asp Gly Met Ala Ser

115 CTA TCT CTG CCA TTA CCC CTT TCG ACC GGC AGA GGG GCT CCT GGA TTA TCG CTG
    GAT AGA GAC GGT AAT GGG GAA AGC TGG CCG TCT CCC CGA GGA CCT AAT AGC GAC
39► Leu Ser Leu Pro Leu Pro Leu Ser Thr Gly Arg Gly Ala Pro Gly Leu Ser Leu

172 ATT TAC AGC AAC AGT GCA GGT AAT GGG CCT TTC GGC ATC TGG CAA TGC GGT GTT
    TAA ATG TCG TTG TCA CGT CCA TTA CCC GGA AAG CCG TAG ACG GTT ACG CCA CAA
58► Ile Tyr Ser Asn Ser Ala Gly Asn Gly Pro Phe Gly Ile Trp Gln Cys Gly Val

229 ATG TCC ATT AGC CGA CCC ACC CAA CAT GGC CTT CAA CAT TGA CGA CGT GCA
    TAC AGG TAA TCG GCT GGG TGG GTT GTA CCG GAA GTT GTA ACT GCT GCA
77► Met Ser Ile Ser Arg Arg Thr Gln His Gly Leu Gln His ••• Arg Arg
                                                        P2.3.5R
```

FIG. 1
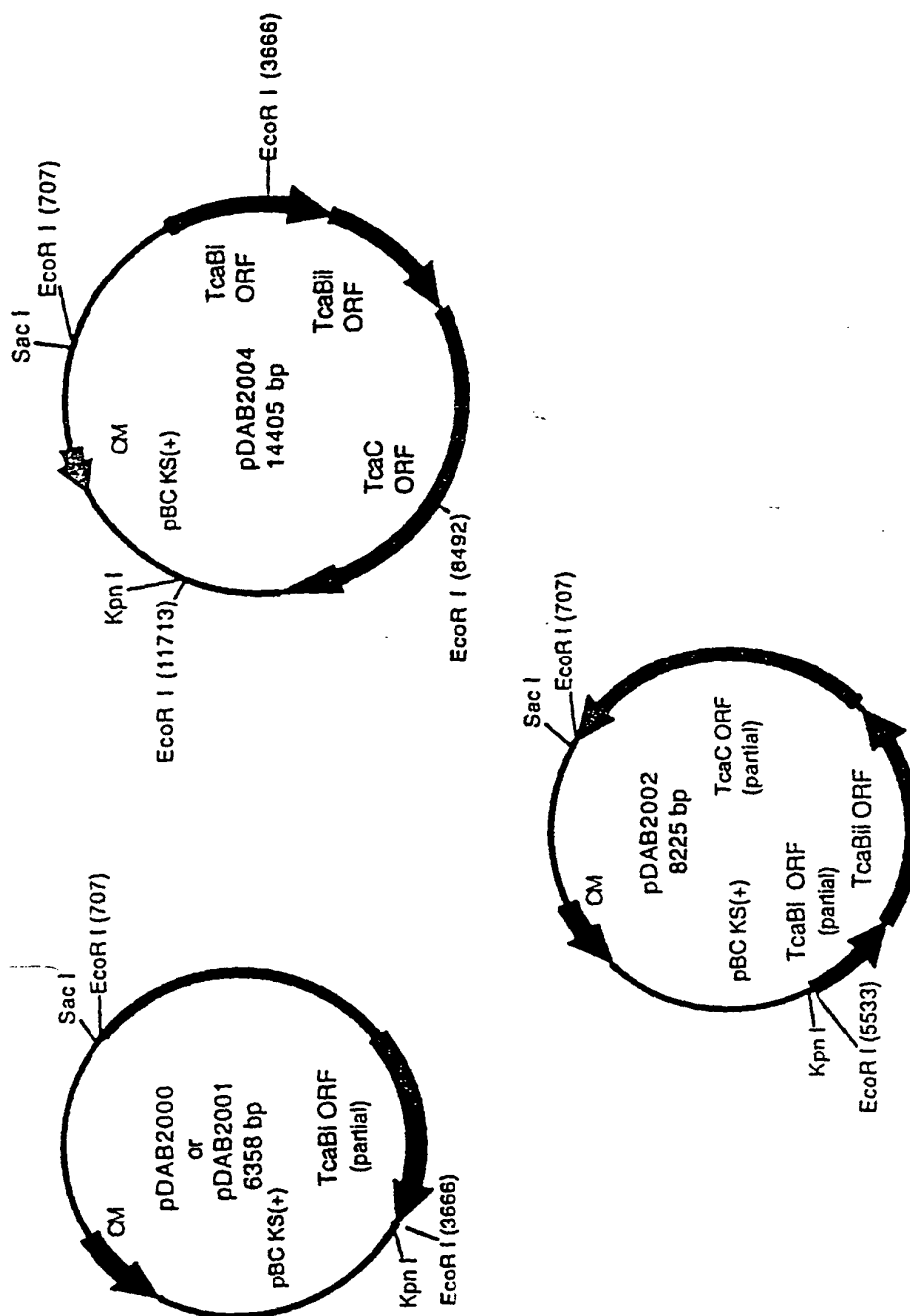
FIG. 2 Plasmids used in sequencing the *tca* locus. CM = Chloramphenicol resistance gene.
ORF = Open Reading Frame

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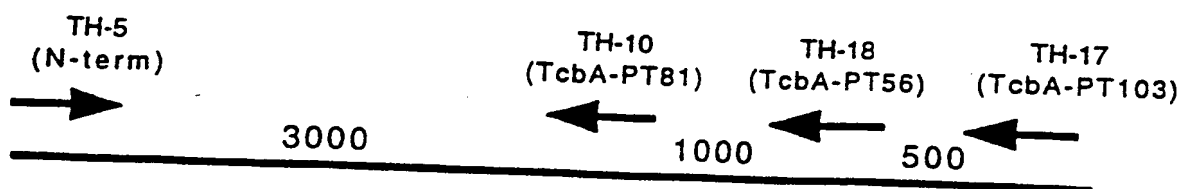
FIG. 3 Physical Map of DNA fragments of *tcb* locus.
Estimated distance between fragments given in nucleotides.

```
              1740       1750       1760       1770       1780
TcbA    SSAQALKNDS EPMDFSGANA LYFWELFYYT PMMMAHRLLQ EQNFDAANHW
                       n
                     |450    |    460   |    470   |    480   |
TcaBi         ____gS nPvDFSGpyg iYlWEiFfhi PflvtvRmqt EQryedAdtW>
              ^^  ^^^^^^^^^v^ ^^^^^^^^-- ^-^^v^^vv ^^-^^-^^v^


              1790       1800       1810       1820       1830
TcbA    FRYVWSPSGY IVDGKIAIYH WNVRPLEEDT SWNAQQLDST DPDAVAQDDP
                rdangql              |          |          |
             490   |         510        520        530        |
TcaBi   ykYifrsaGY ImDGskprY- WNVmPLqlDT aWdttQpatT DPDviAmaDP>
        ^^^--^^^^ ^^^^-v^v^| ^^^-^^^v^^ ^^^^v^v-^^ ^^^-^^v-^^


              1840       1850       1860       1870       1880
TcbA    MHYKVATFMA TLDLLMARGD AAYRQLERDT LAEAKMWYTQ ALNLLGDEPQ
                 |          |          |          |          |
        540        550        560        570        580        |
TcaBi   MHYKlAiFlh TLDLLiARGD sAYRQLERDT LvEAKMyYiQ AqqLLGprPd>
        ^^^^^^-^^v ^^^^^^^^^^ ^^^^^^^^^^ ^-^^^^-^-^ ^v^^^^^vv^^


              1890       1900       1910       1920       1930
TcbA    VMLSTTWANP TLGNAASKTT QQVRQQVLTQ LRLNSRVKTP LLGTANSLTA
                 |
        600      |
TcaBi   ihttnTWpNP TLsk>
        ^vv^-^^^^^ ^^^^


              1940       1950       1960       1970       1980
TcbA    LFLPQENSKL KGYWRTLAQR MFNLRHNLSI DGQPLSLPLY AKPADPKALL
                 |          |          |          |          |
        20        30         40         50         60
TcaBii  _FLPpyNdvL lGYWdkLelR lyNLRHNLSl DGQPLnLPLY AtPvDPKtLq>
        ^^^-v^-v^ v^^^v-^-v^ ^^^^^^^^^^ ^^^^^^^^^^ ^-^-^^^^^v


              1990       2000       2010       2020       2030
TcbA    SAAVSASQGG ADLPKAPLTI HRFPQMLEGA RGLVNQLIQF GSSLLGYSER
                 |          |gw        |          |          |
        70        80        |   90     |   100    |   110    |
TcaBii  rqqaggdgtG sspaggqgsv qRyPllvErA RsaVslLtQF GnSLqttlEh>
        ----^^-v-^ ^-v^v^-v^^ ^^^^v^^^v^ ^^v^v^-^^ ^^^^v-vv^^


              2040       2050       2060       2070       2080
TcbA    QDAEAMSQLL QTQASELILT SIRMQDNQLA ELDSEKTALQ VSLAGVQQRF
                 |          |          |          |          |
        120       |   130   |    140   |    150   |    160   |
TcaBii  QDnEkMtiLL QTQqeailkh qhdiQqNnLk gLqhslTALQ aSrdGdtlRq>
        ^^-^v^^v^^ ^^^---^^vv vvv^^^^^v -^^v-v^^^^ -^v-^vvv^v
```

FIG. 4A

```
                2090      2100      2110      2120      2130
TcbA            DSYSQLYEEN INAGEQRALA LRSESAIESQ GAQISRMAGA GVDMAPNIFG
                                                                a
                170 |     180 |     190 |     200 |     | 210 |
TcaBii          khYSdLingg lsAaEiagLt LRStamI-tn Gvatglliag GinavPNvFG>
                -v^^^^v^-- ^^^^^vv^^^ ^^^-^v^ ^^ ^---^v^v^^ ^^^v-^^^^^

                2140      2150      ─ 2160      2170      2180
TcbA            LADGGMHYGA IAYAIADGIE LSASAKMVDA EKVAQSEIYR RRRQEWKIQR
                220 |     230 |     240 |     250 |     260 |
TcaBii          LAnGGsewGA pligsgqatq vgAgiqdqsA gisevtagYq RRqeEWalQR>
                ^^^^^v^-^^ vvv^v^^^-^ ^^^^^^vv-^ -vv-v^-v^^ ^^^^^^v^^^

                2190      2200      2210      2220      2230
TcbA            DNAQAEINQL NAQLESLSIR REAAEMQKEY LKTQQAQAQA QLTFLRSKFS
                270 |     280 |     290 |     300 |     310 |
TcaBii          DiAdnEItQL dAQiqSLqeq itmAqkQitl seTeQAnAQA iydlqttrFt>
                ^v^-^^-^^ ^^^^^^vv^ v-v^^-^v-v v-^^^^^^^^ vv-^vv^^^^

                2240      2250      2260      2270      2280
TcbA            NQALYSWLRG RLSGIYFQFY DLAVSRCLMA EQSYQWEAND NSISFVKPGA
                                                         l
                320 |     330 |     340 |     |         360 |
TcaBii          gQALYnWmaG RLSalYyQmY DstlpiCLqp kaalvqEgek eSdSlfqvpv>
                -^^^^^^^v^ ^^^^^^^-^ ^v^^^^v^v^ --^vvv^^^- ^^v^^v^vv-

                2290      2300      2310      2320      2330
TcbA            WQGTYAGLLC GEALIQNLAQ MEEAYLKWES RALEVERTVS LAVVYDSLEG
                370 |     380 |     390 |     400 |     410 |
TcaBii          WndlwqGLLa GEgLsseLqk ldaiwLargg igLEaiRTVS Ldtlfgt--G>
                ^^^v--^^^v ^^^^vv^^-^ ^^-v-^v^-^ v^^^-v^^^^ ^--^^^^ ^

                2340      2350      2360      2370      2380
TcbA            NDRFNLAEQI PALLDKGEGT AGTKKNGLSL ANAILSASVK LSDLKLGTDY
                          420 |     430 |     440 |     450 |
TcaBii          ----tLsEnI nkvLn-GEtv spsggvtLaL tgdIfqAtld LSqLgLdnsY>
                -^^^^ vv^^^ ^^-- ^v^vvv-^^^ ^--^^v^^^- ^^^^v^^--^

                2390      2400      2410      2420      2430
TcbA            PDSIVGSNKV RRIKQISVSL PALVGPYQDV QAMLSYGGST QLPKGCSALA
                                                         i
                460 |     470 |     480 |     490 ||    500 |
TcaBii          -n--lGneKk RRIKrIaVtL PtLlGPYQDl eAtLvmGaea aLshGvndgg>
                 ^  ^^^^^v ^^^^^^^^^ ^^^^^^^^^ ^^v^vv^^-^ -^^-^v^-v^

                2440      2450      2460      2470
TcbA            VSHGTNDSGQ FQLDFNDGKY LPFEGIALDD QGTLNLQFPN
                510 |     520 |     530 |              |
TcaBii          rfvtdfndsr F-LpF-eGrd attgtleLn>
                vvv--v^-^^ ^ ^v^ ^^^v v-v--^-^^|
```
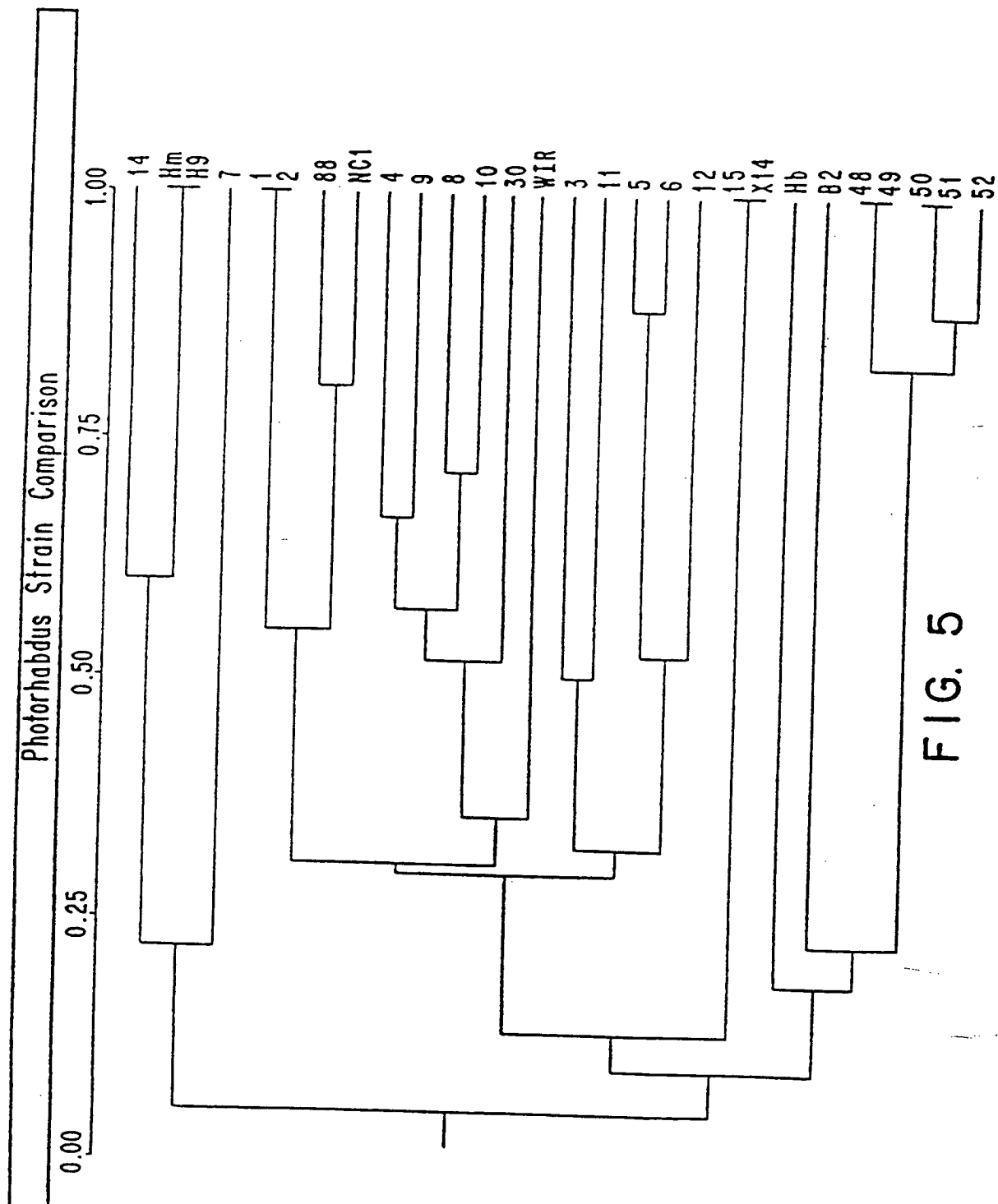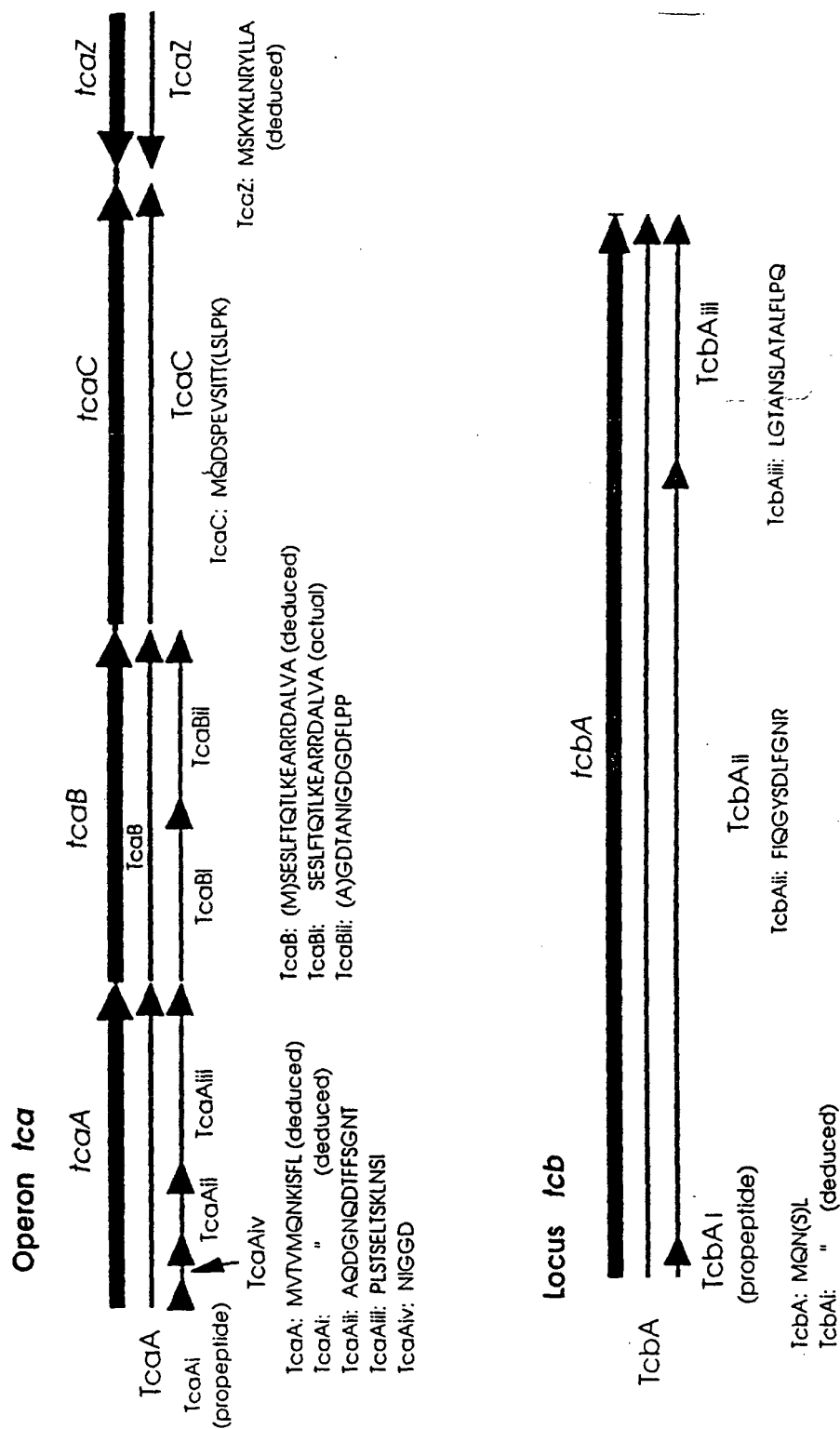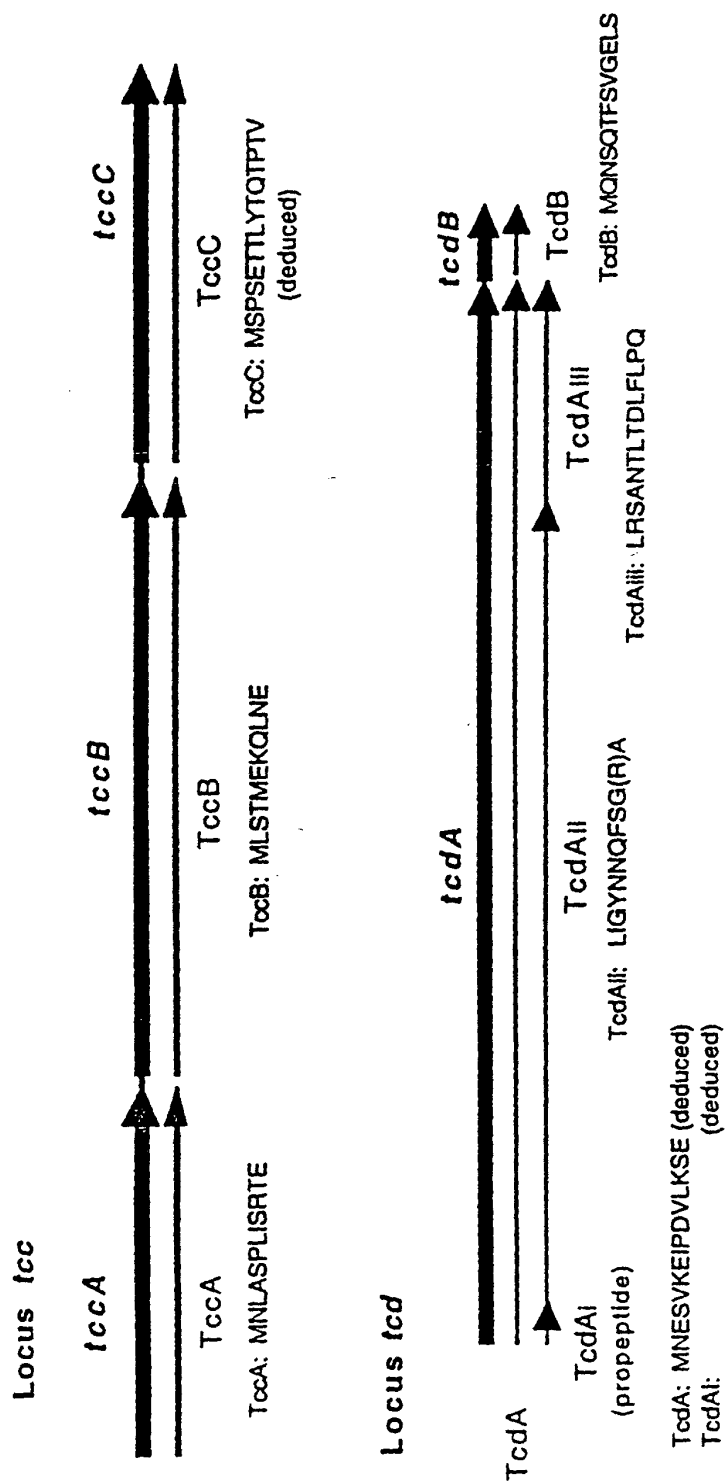
FIG. 4B

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Photorhabdus Strain Comparison

FIG. 5

FIG.6A loci *tca* and *tcb*, primary gene products, and derived peptides

FIG. 6B Loci *tcc* and *tcd*, primary gene products, and derived peptides.